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(54) Title: PATHOGEN TOLERANCE GENES

(57) Abstract: The present invention relates to transgenic plants and methods of making transgenic plant using punitive transcription factors that modulate the transgenic plant's susceptibility to disease.

PATHOGEN TOLERANCE GENES

RELATED APPLICATION INFORMATION

The present invention claims the benefit from US Provisional Patent Application Serial Nos. 60/166,228 filed November 17, 1999 and 60/197,899 filed April 17, 2000 and "Plant Trait Modification III" filed August 22, 2000.

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FIELD OF THE INVENTION

This invention relates to the field of plant biology. More particularly, the present invention pertains to compositions and methods for phenotypically modifying a plant.

BACKGROUND OF THE INVENTION

Transcription factors can modulate gene expression, either increasing or decreasing (inducing or repressing) the rate of transcription. This modulation results in differential levels of gene expression at various developmental stages, in different tissues and cell types, and in response to different exogenous (e.g., environmental) and endogenous stimuli throughout the life cycle of the organism.

Because transcription factors are key controlling elements of biological pathways, altering the expression levels of one or more transcription factors can change entire biological pathways in an organism. For example, manipulation of the levels of selected transcription factors may result in increased expression of economically useful proteins or metabolic chemicals in plants or to improve other agriculturally relevant characteristics. Conversely, blocked or reduced expression of a transcription factor may reduce biosynthesis of unwanted compounds or remove an undesirable trait. Therefore, manipulating transcription factor levels in a plant offers tremendous potential in agricultural biotechnology for modifying a plant's traits.

The present invention provides novel transcription factors useful for modifying a plant's phenotype in desirable ways, such as modifying a plant's pathogen tolerance.

SUMMARY OF THE INVENTION

In a first aspect, the invention relates to a recombinant polynucleotide comprising a nucleotide sequence selected from the group consisting of: (a) a nucleotide sequence encoding a polypeptide comprising a sequence selected from SEQ ID Nos. 2N, where N=1-29, or a complementary nucleotide sequence thereof; (b) a nucleotide sequence encoding a polypeptide comprising a conservatively substituted variant of a polypeptide of (a); (c) a nucleotide sequence comprising a sequence selected from those of SEQ ID Nos. 2N-1, where N=1-29, or a complementary nucleotide sequence thereof; (d) a nucleotide sequence

comprising silent substitutions in a nucleotide sequence of (c); (e) a nucleotide sequence which hybridizes under stringent conditions over substantially the entire length of a nucleotide sequence of one or more of: (a), (b), (c), or (d); (f) a nucleotide sequence comprising at least 15 consecutive nucleotides of a sequence of any of (a)-(e); (g) a nucleotide sequence comprising a subsequence or fragment of any of (a)-(f), which subsequence or fragment encodes a polypeptide having a biological activity that modifies a plant's pathogen tolerance; (h) a nucleotide sequence having at least 31% sequence identity to a nucleotide sequence of any of (a)-(g); (i) a nucleotide sequence having at least 60% identity sequence identity to a nucleotide sequence of any of (a)-(g); (j) a nucleotide sequence which encodes a polypeptide having at least 31% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-29; (k) a nucleotide sequence which encodes a polypeptide having at least 60% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-29; and (1) a nucleotide sequence which encodes a conserved domain of a polypeptide having at least 65% sequence identity to a conserved domain of a polypeptide of SEQ ID Nos. 2N, where N=1-29. The recombinant polynucleotide may further comprise a constitutive, inducible, or tissue-active promoter operably linked to the nucleotide sequence. The invention also relates to compositions comprising at least two of the above described polynucleotides.

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In a second aspect, the invention is an isolated or recombinant polypeptide comprising a subsequence of at least about 15 contiguous amino acids encoded by the recombinant or isolated polynucleotide described above.

In another aspect, the invention is a transgenic plant comprising one or more of the above described recombinant polynucleotides. In yet another aspect, the invention is a plant with altered expression levels of a polynucleotide described above or a plant with altered expression or activity levels of an above described polypeptide. Further, the invention may be a plant lacking a nucleotide sequence encoding a polypeptide comprising a sequence selected from SEQ ID Nos. 2N, where N=1-29.

The plant may be a soybean, wheat, corn, potato, cotton, rice, oilseed rape, sunflower, alfalfa, sugarcane, turf, banana, blackberry, blueberry, strawberry, raspberry, cantaloupe, carrot, cauliflower, coffee, cucumber, eggplant, grapes, honeydew, lettuce, mango, melon, onion, papaya, peas, peppers, pineapple, spinach, squash, sweet corn, tobacco, tomato, watermelon, rosaceous fruits, or vegetable brassicas plant.

In a further aspect, the invention relates to a cloning or expression vector comprising the isolated or recombinant polynucleotide described above or cells comprising the cloning or expression vector.

In yet a further aspect, the invention relates to a composition produced by incubating a polynucleotide of the invention with a nuclease, a restriction enzyme, a polymerase; a polymerase and a primer; a cloning vector, or with a cell.

Furthermore, the invention relates to a method for producing a plant having improved pathogen tolerance. The method comprises altering the expression of an isolated or recombinant polynucleotide of the invention or altering the expression or activity of a polypeptide of the invention in a plant to produce a modified plant, and selecting the modified plant for modified pathogen tolerance.

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In another aspect, the invention relates to a method of identifying a factor that is modulated by or interacts with a polypeptide encoded by a polynucleotide of the invention. The method comprises expressing a polypeptide encoded by the polynucleotide in a plant; and identifying at least one factor that is modulated by or interacts with the polypeptide. In one embodiment the method for identifying modulating or interacting factors is by detecting binding by the polypeptide to a promoter sequence, or by detecting interactions between an additional protein and the polypeptide in a yeast two hybrid system, or by detecting expression of a factor by hybridization to a microarray, subtractive hybridization or differential display.

In yet another aspect, the invention is a method of identifying a molecule that modulates activity or expression of a polynucleotide or polypeptide of interest. The method comprises placing the molecule in contact with a plant comprising the polynucleotide or polypeptide encoded by the polynucleotide of the invention and monitoring one or more of the expression level of the polynucleotide in the plant, the expression level of the polypeptide in the plant, and modulation of an activity of the polypeptide in the plant.

In yet another aspect, the invention relates to an integrated system, computer or computer readable medium comprising one or more character strings corresponding to a polynucleotide of the invention, or to a polypeptide encoded by the polynucleotide. The integrated system, computer or computer readable medium may comprise a link between one or more sequence strings to a modified plant pathogen tolerance phenotype.

In yet another aspect, the invention is a method for identifying a sequence similar or homologous to one or more polynucleotides of the invention, or one or more polypeptides encoded by the polynucleotides. The method comprises providing a sequence database; and, querying the sequence database with one or more target sequences corresponding to the one or more polynucleotides or to the one or more polypeptides to identify one or more sequence members of the database that display sequence similarity or homology to one or more of the one or more target sequences.

The method may further comprise of linking the one or more of the polynucleotides of the invention, or encoded polypeptides, to a modified plant pathogen tolerance phenotype.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 provides a table of exemplary polynucleotide and polypeptide sequences of the invention. The table includes from left to right for each sequence: the SEQ ID No., the internal code reference number (GID), whether the sequence is a polynucleotide or polypeptide sequence, and identification of any conserved domains for the polypeptide sequences.

Figure 2 provides a table of exemplary sequences that are homologous to other sequences provided in the Sequence Listing and that are derived from Arabidopsis thaliana. The table includes from left to right: the SEQ ID No., the internal code reference number (GID), identification of the homologous sequence, whether the sequence is a polynucleotide or polypeptide sequence, and identification of any conserved domains for the polypeptide sequences.

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Figure 3 provides a table of exemplary sequences that are homologous to the sequences provided in Figures 1 and 2 and that are derived from plants other than *Arabidopsis thaliana*. The table includes from left to right: the SEQ ID No., the internal code reference number (GID), the unique GenBank sequence ID No. (NID), the probability that the comparison was generated by chance (P-value), and the species from which the homologous gene was identified.

DETAILED DESCRIPTION

The present invention relates to polynucleotides and polypeptides, e.g. for modifying phenotypes of plants.

In particular, the polynucleotides or polypeptides are useful for modifying traits associated with a plant's pathogen tolerance when the expression levels of the polynucleotides or expression levels or activity levels of the polypeptides are altered. Specifically, the polynucleotides and polypeptides are useful for modifying traits associated with a plant's pathogen tolerance, such as alterations in cell wall composition, trichome number or structure, callose induction, phytoalexin induction, alterations in the cell death response, or the like. Transgenic plants employing the polynucleotides or polypeptides of the invention are more tolerant to biotrophic or necrotrophic pathogens such as fungi, bacteria, mollicutes, viruses, nematodes, parasitic higher plants or the like.

The polynucleotides of the invention encode plant transcription factors. The plant transcription factors are derived, e.g., from *Arabidopsis thaliana* and can belong, e.g., to one or more of the following transcription factor families: the AP2 (APETALA2) domain transcription factor family (Riechmann and Meyerowitz (1998) <u>J. Biol. Chem.</u> 379:633-646); the MYB transcription factor family (Martin and Paz-Ares (1997) <u>Trends Genet</u>, 13:67-73); the MADS domain transcription factor family (Riechmann and Meyerowitz (1997) <u>J. Biol.</u>

Chem. 378:1079-1101); the WRKY protein family (Ishiguro and Nakamura (1994) Mol. Gen. Genet. 244:563-571); the ankyrin-repeat protein family (Zhang et al. (1992) Plant Cell 4:1575-1588); the miscellaneous protein (MISC) family (Kim et al. (1997) Plant J. 11:1237-1251); the zinc finger protein (Z) family (Klug and Schwabe (1995) FASEB J. 9: 597-604); the homeobox (HB) protein family (Duboule (1994) Guidebook to the Homeobox Genes. Oxford University Press); the CAAT-element binding proteins (Forsburg and Guarente (1989) Genes Dev. 3:1166-1178); the squamosa promoter binding proteins (SPB) (Klein et al. (1996) Mol. Gen. Genet. 1996 250:7-16); the NAM protein family; the IAA/AUX proteins (Rouse et al. (1998) Science 279:1371-1373); the HLH/MYC protein family (Littlewood et al. (1994) Prot. Profile 1:639-709); the DNA-binding protein (DBP) family (Tucker et al. (1994) EMBO J. 13:2994-3002); the bZIP family of transcription factors (Foster et al. (1994) FASEB J. 8:192-200); the BPF-1 protein (Box P-binding factor) family (da Costa e Silva et al. (1993) Plant J. 4:125-135); and the golden protein (GLD) family (Hall et al. (1998) Plant Cell 10:925-936).

In addition to methods for modifying a plant phenotype by employing one or more polynucleotides and polypeptides of the invention described herein, the polynucleotides and polypeptides of the invention have a variety of additional uses. These uses include their use in the recombinant production (i.e, expression) of proteins; as regulators of plant gene expression, as diagnostic probes for the presence of complementary or partially complementary nucleic acids (including for detection of natural coding nucleic acids); as substrates for further reactions, e.g., mutation reactions, PCR reactions, or the like, of as substrates for cloning e.g., including digestion or ligation reactions, and for identifying exogenous or endogenous modulators of the transcription factors.

DEFINITIONS

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A "polynucleotide" is a nucleic acid sequence comprising a plurality of polymerized nucleotide residues, e.g., at least about 15 consecutive polymerized nucleotide residues, optionally at least about 30 consecutive nucleotides, at least about 50 consecutive nucleotides. In many instances, a polynucleotide comprises a nucleotide sequence encoding a polypeptide (or protein) or a domain or fragment thereof. Additionally, the polynucleotide may comprise a promoter, an intron, an enhancer region, a polyadenylation site, a translation initiation site, 5' or 3' untranslated regions, a reporter gene, a selectable marker, or the like. The polynucleotide can be single stranded or double stranded DNA or RNA. The polynucleotide optionally comprises modified bases or a modified backbone. The polynucleotide can be, e.g., genomic DNA or RNA, a transcript (such as an mRNA), a cDNA, a PCR product, a cloned DNA, a synthetic DNA or RNA, or the like. The polynucleotide can comprise a sequence in either sense or antisense orientations.

A "recombinant polynucleotide" is a polynucleotide that is not in its native state, e.g., the polynucleotide comprises a nucleotide sequence not found in nature, or the polynucleotide is in a context other than that in which it is naturally found, e.g., separated from nucleotide sequences with which it typically is in proximity in nature, or adjacent (or contiguous with) nucleotide sequences with which it typically is not in proximity. For example, the sequence at issue can be cloned into a vector, or otherwise recombined with one or more additional nucleic acid.

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An "isolated polynucleotide" is a polynucleotide whether naturally occurring or recombinant, that is present outside the cell in which it is typically found in nature, whether purified or not. Optionally, an isolated polynucleotide is subject to one or more enrichment or purification procedures, e.g., cell lysis, extraction, centrifugation, precipitation, or the like.

A "recombinant polypeptide" is a polypeptide produced by translation of a recombinant polynucleotide. An "isolated polypeptide," whether a naturally occurring or a recombinant polypeptide, is more enriched in (or out of) a cell than the polypeptide in its natural state in a wild type cell, e.g., more than about 5% enriched, more than about 10% enriched, or more than about 20%, or more than about 50%, or more, enriched, i.e., alternatively denoted: 105%, 110%, 120%, 150% or more, enriched relative to wild type standardized at 100%. Such an enrichment is not the result of a natural response of a wild type plant. Alternatively, or additionally, the isolated polypeptide is separated from other cellular components with which it is typically associated, e.g., by any of the various protein purification methods herein.

The term "transgenic plant" refers to a plant that contains genetic material, not found in a wild type plant of the same species, variety or cultivar. The genetic material may include a transgene, an insertional mutagenesis event (such as by transposon or T-DNA insertional mutagenesis), an activation tagging sequence, a mutated sequence, a homologous recombination event or a sequence modified by chimeraplasty. Typically, the foreign genetic material has been introduced into the plant by human manipulation.

A transgenic plant may contain an expression vector or cassette. The expression cassette typically comprises a polypeptide-encoding sequence operably linked (i.e., under regulatory control of) to appropriate inducible or constitutive regulatory sequences that allow for the expression of polypeptide. The expression cassette can be introduced into a plant by transformation or by breeding after transformation of a parent plant. A plant refers to a whole plant as well as to a plant part, such as seed, fruit, leaf, or root, plant tissue, plant cells or any other plant material, e.g., a plant explant, as well as to progeny thereof, and to in vitro systems that mimic biochemical or cellular components or processes in a cell.

The phrase "ectopically expression or altered expression" in reference to a polynucleotide indicates that the pattern of expression in, e.g., a transgenic plant or plant

tissue, is different from the expression pattern in a wild type plant or a reference plant of the same species. For example, the polynucleotide or polypeptide is expressed in a cell or tissue type other than a cell or tissue type in which the sequence is expressed in the wild type plant, or by expression at a time other than at the time the sequence is expressed in the wild type plant, or by a response to different inducible agents, such as hormones or environmental signals, or at different expression levels (either higher or lower) compared with those found in a wild type plant. The term also refers to altered expression patterns that are produced by lowering the levels of expression to below the detection level or completely abolishing expression. The resulting expression pattern can be transient or stable, constitutive or inducible. In reference to a polypeptide, the term "ectopic expression or altered expression" further may relate to altered activity levels resulting from the interactions of the polypeptides with exogenous or endogenous modulators or from interactions with factors or as a result of the chemical modification of the polypeptides.

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The term "fragment" or "domain," with respect to a polypeptide, refers to a subsequence of the polypeptide. In some cases, the fragment or domain, is a subsequence of the polypeptide which performs at least one biological function of the intact polypeptide in substantially the same manner, or to a similar extent, as does the intact polypeptide. For example, a polypeptide fragment can comprise a recognizable structural motif or functional domain such as a DNA binding domain that binds to a DNA promoter region, an activation domain or a domain for protein-protein interactions. Fragments can vary in size from as few as 6 amino acids to the full length of the intact polypeptide, but are preferably at least about 30 amino acids in length and more preferably at least about 60 amino acids in length. In reference to a nucleotide sequence, "a fragment" refers to any subsequence of a polynucleotide, typically, of at least consecutive about 15 nucleotides, preferably at least about 30 nucleotides, more preferably at least about 50, of any of the sequences provided herein.

The term "trait" refers to a physiological, morphological, biochemical or physical characteristic of a plant or particular plant material or cell. In some instances, this characteristic is visible to the human eye, such as seed or plant size, or can be measured by available biochemical techniques, such as the protein, starch or oil content of seed or leaves or by the observation of the expression level of genes, e.g., by employing Northern analysis, RT-PCR, microarray gene expression assays or reporter gene expression systems, or by agricultural observations such as stress tolerance, yield or pathogen tolerance.

"Trait modification" refers to a detectable difference in a characteristic in a plant ectopically expressing a polynucleotide or polypeptide of the present invention relative to a plant not doing so, such as a wild type plant. In some cases, the trait modification can be evaluated quantitatively. For example, the trait modification can entail at least about a 2%

increase or decrease in an observed trait (difference), at least a 5% difference, at least about a 10% difference, at least about a 20% difference, at least about a 30%, at least about a 50%, at least about a 70%, or at least about a 100%, or an even greater difference. It is known that there can be a natural variation in the modified trait. Therefore, the trait modification observed entails a change of the normal distribution of the trait in the plants compared with the distribution observed in wild type plant.

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Trait modifications of particular interest include those to seed (such as embryo or endosperm), fruit, root, flower, leaf, stem, shoot, seedling or the like, including: enhanced tolerance to environmental conditions including freezing, chilling, heat, drought, water saturation, radiation and ozone; improved tolerance to microbial, fungal or viral diseases; improved tolerance to pest infestations, including nematodes, mollicutes, parasitic higher plants or the like; decreased herbicide sensitivity; improved tolerance of heavy metals or enhanced ability to take up heavy metals; improved growth under poor photoconditions (e.g., low light and/or short day length), or changes in expression levels of genes of interest. Other phenotype that can be modified relate to the production of plant metabolites, such as variations in the production of taxol, tocopherol, tocotrienol, sterols, phytosterols, vitamins, wax monomers, anti-oxidants, amino acids, lignins, cellulose, tannins, prenyllipids (such as chlorophylls and carotenoids), glucosinolates, and terpenoids, enhanced or compositionally altered protein or oil production (especially in seeds), or modified sugar (insoluble or soluble) and/or starch composition. Physical plant characteristics that can be modified include cell development (such as the number of trichomes), fruit and seed size and number, yields of plant parts such as stems, leaves and roots, the stability of the seeds during storage, characteristics of the seed pod (e.g., susceptibility to shattering), root hair length and quantity, internode distances, or the quality of seed coat. Plant growth characteristics that can be modified include growth rate, germination rate of seeds, vigor of plants and seedlings, leaf and flower senescence, male sterility, apomixis, flowering time, flower abscission, rate of nitrogen uptake, biomass or transpiration characteristics, as well as plant architecture characteristics such as apical dominance, branching patterns, number of organs, organ identity, organ shape or size.

POLYPEPTIDES AND POLYNUCLEOTIDES OF THE INVENTION

The present invention provides, among other things, transcription factors (TFs), and transcription factor homologue polypeptides, and isolated or recombinant polynucleotides encoding the polypeptides. These polypeptides and polynucleotides may be employed to modify a plant's pathogen tolerance.

Exemplary polynucleotides encoding the polypeptides of the invention were identified in the *Arabidopsis thaliana* GenBank database using publicly available sequence

analysis programs and parameters. Sequences initially identified were then further characterized to identify sequences comprising specified sequence strings corresponding to sequence motifs present in families of known transcription factors. Polynucleotide sequences meeting such criteria were confirmed as transcription factors.

Additional polynucleotides of the invention were identified by screening Arabidopsis thaliana and/or other plant cDNA libraries with probes corresponding to known transcription factors under low stringency hybridization conditions. Additional sequences, including full length coding sequences were subsequently recovered by the rapid amplification of cDNA ends (RACE) procedure, using a commercially available kit according to the manufacturer's instructions. Where necessary, multiple rounds of RACE are performed to isolate 5' and 3' ends. The full length cDNA was then recovered by a routine end-to-end polymerase chain reaction (PCR) using primers specific to the isolated 5' and 3' ends. Exemplary sequences are provided in the Sequence Listing.

The polynucleotides of the invention were ectopically expressed in overexpressor or knockout plants and changes in the pathogen tolerance of the plants was observed. Therefore, the polynucleotides and polypeptides can be employed to improve the pathogen resistance of plants.

Making polynucleotides

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The polynucleotides of the invention include sequences that encode transcription factors and transcription factor homologue polypeptides and sequences complementary thereto, as well as unique fragments of coding sequence, or sequence complementary thereto. Such polynucleotides can be, e.g., DNA or RNA, e.g., mRNA, cRNA, synthetic RNA, genomic DNA, cDNA synthetic DNA, oligonucleotides, etc. The polynucleotides are either double-stranded or single-stranded, and include either, or both sense (i.e., coding) sequences and antisense (i.e., non-coding, complementary) sequences. The polynucleotides include the coding sequence of a transcription factor, or transcription factor homologue polypeptide, in isolation, in combination with additional coding sequences (e.g., a purification tag, a localization signal, as a fusion-protein, as a pre-protein, or the like), in combination with non-coding sequences (e.g., introns or inteins, regulatory elements such as promoters, enhancers, terminators, and the like), and/or in a vector or host environment in which the polynucleotide encoding a transcription factor or transcription factor homologue polypeptide is an endogenous or exogenous gene.

A variety of methods exist for producing the polynucleotides of the invention. Procedures for identifying and isolating DNA clones are well known to those of skill in the art, and are described in, e.g., Berger and Kimmel, <u>Guide to Molecular Cloning Techniques</u>, <u>Methods in Enzymology</u> volume 152 Academic Press, Inc., San Diego, CA ("Berger");

Sambrook et al., Molecular Cloning - A Laboratory Manual (2nd Ed.), Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989 ("Sambrook") and Current Protocols in Molecular Biology, F.M. Ausubel et al., eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (supplemented through 2000) ("Ausubel").

Alternatively, polynucleotides of the invention, can be produced by a variety of in vitro amplification methods adapted to the present invention by appropriate selection of specific or degenerate primers. Examples of protocols sufficient to direct persons of skill through in vitro amplification methods, including the polymerase chain reaction (PCR) the ligase chain reaction (LCR), Qbeta-replicase amplification and other RNA polymerase mediated techniques (e.g., NASBA), e.g., for the production of the homologous nucleic acids of the invention are found in Berger, Sambrook, and Ausubel, as well as Mullis et al., (1987) PCR Protocols A Guide to Methods and Applications (Innis et al. eds) Academic Press Inc. San Diego, CA (1990) (Innis). Improved methods for cloning in vitro amplified nucleic acids are described in Wallace et al., U.S. Pat. No. 5,426,039. Improved methods for amplifying large nucleic acids by PCR are summarized in Cheng et al. (1994) Nature 369: 684-685 and the references cited therein, in which PCR amplicons of up to 40kb are generated. One of skill will appreciate that essentially any RNA can be converted into a double stranded DNA suitable for restriction digestion, PCR expansion and sequencing using reverse transcriptase and a polymerase. See, e.g., Ausubel, Sambrook and Berger, all supra.

Alternatively, polynucleotides and oligonucleotides of the invention can be assembled from fragments produced by solid-phase synthesis methods. Typically, fragments of up to approximately 100 bases are individually synthesized and then enzymatically or chemically ligated to produce a desired sequence, e.g., a polynucletotide encoding all or part of a transcription factor. For example, chemical synthesis using the phosphoramidite method is described, e.g., by Beaucage et al. (1981) <u>Tetrahedron Letters</u> 22:1859-69; and Matthes et al. (1984) <u>EMBO J.</u> 3:801-5. According to such methods, oligonucleotides are synthesized, purified, annealed to their complementary strand, ligated and then optionally cloned into suitable vectors. And if so desired, the polynucleotides and polypeptides of the invention can be custom ordered from any of a number of commercial suppliers.

HOMOLOGOUS SEQUENCES

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Sequences homologous, i.e., that share significant sequence identity or similarity, to those provided in the Sequence Listing, derived from Arabidopsis thaliana or from other plants of choice are also an aspect of the invention. Homologous sequences can be derived from any plant including monocots and dicots and in particular agriculturally important plant species, including but not limited to, crops such as soybean, wheat, corn,

potato, cotton, rice, oilseed rape (including canola), sunflower, alfalfa, sugarcane and turf; or fruits and vegetables, such as banana, blackberry, blueberry, strawberry, and raspberry, cantaloupe, carrot, cauliflower, coffee, cucumber, eggplant, grapes, honeydew, lettuce, mango, melon, onion, papaya, peas, peppers, pineapple, spinach, squash, sweet corn, tobacco, tomato, watermelon, rosaceous fruits (such as apple, peach, pear, cherry and plum) and vegetable brassicas (such as broccoli, cabbage, cauliflower, brussel sprouts and kohlrabi). Other crops, fruits and vegetables whose phenotype can be changed include barley, rye, millet, sorghum, currant, avocado, citrus fruits such as oranges, lemons, grapefruit and tangerines, artichoke, cherries, nuts such as the walnut and peanut, endive, leek, roots, such as arrowroot, beet, cassava, turnip, radish, yam, and sweet potato, and beans. The homologous sequences may also be derived from woody species, such pine, poplar and eucalyptus.

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Transcription factors that are homologous to the listed sequences will typically share at least about 31% amino acid sequence identity. More closely related transcription factors can share at least about 50%, about 60%, about 65%, about 70%, about 75% or about 80% or about 90% or about 95% or about 98% or more sequence identity with the listed sequences. Factors that are most closely related to the listed sequences share, e.g., at least about 85%, about 90% or about 95% or more % sequence identity to the listed sequences. At the nucleotide level, the sequences will typically share at least about 40% nucleotide sequence identity, preferably at least about 50%, about 60%, about 70% or about 80% sequence identity, and more preferably about 85%, about 90%, about 95% or about 97% or more sequence identity to one or more of the listed sequences. The degeneracy of the genetic code enables major variations in the nucleotide sequence of a polynucleotide while maintaining the amino acid sequence of the encoded protein. Conserved domains within a transcription factor family may exhibit a higher degree of sequence homology, such as at least 65% sequence identity including conservative substitutions, and preferably at least 80% sequence identity.

Identifying Nucleic Acids by Hybridization

Polynucleotides homologous to the sequences illustrated in the Sequence Listing can be identified, e.g., by hybridization to each other under stringent or under highly stringent conditions. Single stranded polynucleotides hybridize when they associate based on a variety of well characterized physico-chemical forces, such as hydrogen bonding, solvent exclusion, base stacking and the like. The stringency of a hybridization reflects the degree of sequence identity of the nucleic acids involved, such that the higher the stringency, the more similar are the two polynucleotide strands. Stringency is influenced by a variety of factors, including temperature, salt concentration and composition, organic and non-organic additives, solvents, etc. present in both the hybridization and wash solutions and incubations (and number), as described in more detail in the references cited above.

An example of stringent hybridization conditions for hybridization of complementary nucleic acids which have more than 100 complementary residues on a filter in a Southern or northern blot is about 5°C to 20°C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Nucleic acid molecules that hybridize under stringent conditions will typically hybridize to a probe based on either the entire cDNA or selected portions, e.g., to a unique subsequence, of the cDNA under wash conditions of 0.2x SSC to 2.0 x SSC, 0.1% SDS at 50-65° C, for example 0.2 x SSC, 0.1% SDS at 65° C. For identification of less closely related homologues washes can be performed at a lower temperature, e.g., 50° C. In general, stringency is increased by raising the wash temperature and/or decreasing the concentration of SSC.

As another example, stringent conditions can be selected such that an oligonucleotide that is perfectly complementary to the coding oligonucleotide hybridizes to the coding oligonucleotide with at least about a 5-10x higher signal to noise ratio than the ratio for hybridization of the perfectly complementary oligonucleotide to a nucleic acid encoding a transcription factor known as of the filing date of the application. Conditions can be selected such that a higher signal to noise ratio is observed in the particular assay which is used, e.g., about 15x, 25x, 35x, 50x or more. Accordingly, the subject nucleic acid hybridizes to the unique coding oligonucleotide with at least a 2x higher signal to noise ratio as compared to hybridization of the coding oligonucleotide to a nucleic acid encoding known polypeptide. Again, higher signal to noise ratios can be selected, e.g., about 5x, 10x, 25x, 35x, 50x or more. The particular signal will depend on the label used in the relevant assay, e.g., a fluorescent label, a colorimetric label, a radio active label, or the like.

Alternatively, transcription factor homologue polypeptides can be obtained by screening an expression library using antibodies specific for one or more transcription factors. With the provision herein of the disclosed transcription factor, and transcription factor homologue nucleic acid sequences, the encoded polypeptide(s) can be expressed and purified in a heterologous expression system (e.g., E. coli) and used to raise antibodies (monoclonal or polyclonal) specific for the polypeptide(s) in question. Antibodies can also be raised against synthetic peptides derived from transcription factor, or transcription factor homologue, amino acid sequences. Methods of raising antibodies are well known in the art and are described in Harlow and Lane (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, New York. Such antibodies can then be used to screen an expression library produced from the plant from which it is desired to clone additional transcription

factor homologues, using the methods described above. The selected cDNAs can be confirmed by sequencing and enzymatic activity.

SEQUENCE VARIATIONS

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It will readily be appreciated by those of skill in the art, that any of a variety of polynucleotide sequences are capable of encoding the transcription factors and transcription factor homologue polypeptides of the invention. Due to the degeneracy of the genetic code, many different polynucleotides can encode identical and/or substantially similar polypeptides in addition to those sequences illustrated in the Sequence Listing.

For example, Table 1 illustrates, e.g., that the codons AGC, AGT, TCA, TCC, TCG, and TCT all encode the same amino acid: serine. Accordingly, at each position in the sequence where there is a codon encoding serine, any of the above trinucleotide sequences can be used without altering the encoded polypeptide.

Table 1

Amino acids			Codon	-				
Alanine	Ala		CCA	666	000	COLL		
		A	GCA	GCC	GCG	GCU		
Cysteine	Cys	С	TGC	TGT				
Aspartic acid	Asp	D	GAC	GAT				
Glutamic acid	Glu	E	GAA	GAG			•	
Phenylalanine	Phe	F	TTC	TTT				
Glycine	Gly	G	GGA	GGC	GGG	GGT		
Histidine	His	H	CAC	CAT				
Isoleucine	Ile	I	ATA	ATC	ATT			
Lysine	Lys	K	AAA -	AAG				
Leucine	Leu	L	TTA	TTG	CTA	CTC	CTG	CTT
Methionine	Met	M	ATG					
Asparagine	Asn	N	AAC	AAT			•	i
Proline	Pro	P	CCA	CCC	CCG	CCT		
Glutamine	Gln	Q	CAA	CAG				
Arginine	Arg	R	AGA	AGG	CGA	CGC	CGG	CGT
Serine	Ser	S	AGC	AGT	TCA	TCC	TCG	TCT
Threonine	Thr	T	ACA	ACC	ACG	ACT		
Valine	Val	V	GTA	GTC	GTG	GTT		l
Tryptophan	Trp	W	TGG					Ì
Tyrosine	Tyr	Y	TAC	TAT				

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Sequence alterations that do not change the amino acid sequence encoded by the polynucleotide are termed "silent" variations. With the exception of the codons ATG and TGG, encoding methionine and tryptophan, respectively, any of the possible codons for the same amino acid can be substituted by a variety of techniques, e.g., site-directed mutagenesis, available in the art. Accordingly, any and all such variations of a sequence selected from the above table are a feature of the invention.

In addition to silent variations, other conservative variations that alter one, or a few amino acids in the encoded polypeptide, can be made without altering the function of the polypeptide, these conservative variants are, likewise, a feature of the invention.

For example, substitutions, deletions and insertions introduced into the sequences provided in the Sequence Listing are also envisioned by the invention. Such sequence modifications can be engineered into a sequence by site-directed mutagenesis (Wu (ed.) Meth. Enzymol. (1993) vol. 217, Academic Press) or the other methods noted below. Amino acid substitutions are typically of single residues; insertions usually will be on the order of about from 1 to 10 amino acid residues; and deletions will range about from 1 to 30 residues. In preferred embodiments, deletions or insertions are made in adjacent pairs, e.g., a deletion of two residues or insertion of two residues. Substitutions, deletions, insertions or any combination thereof can be combined to arrive at a sequence. The mutations that are made in the polynucleotide encoding the transcription factor should not place the sequence out of reading frame and should not create complementary regions that could produce secondary mRNA structure. Preferably, the polypeptide encoded by the DNA performs the desired function.

Conservative substitutions are those in which at least one residue in the amino acid sequence has been removed and a different residue inserted in its place. Such substitutions generally are made in accordance with the Table 2 when it is desired to maintain the activity of the protein. Table 2 shows amino acids which can be substituted for an amino acid in a protein and which are typically regarded as conservative substitutions.

Table 2

•				
Residue	Conservative Substitutions			
Ala	Ser			
Arg	Lys			
Asn	Gln; His			
Asp	Glu			
Gln	Asn			
Cys	Ser			
Glu	Asp			
Gly	Pro			
His	Asn; Gln			
Пе	Leu, Val			
Leu	Ile; Val			
Lys	Arg; Gln			
Met	Leu; Ile			
Phe	Met; Leu; Tyr			
Ser	Thr; Gly			
Thr	Ser;Val			
Ттр	Tyr			
Тут	Trp; Phe			
Val	Ile; Leu			

Substitutions that are less conservative than those in Table 2 can be selected by picking residues that differ more significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. The substitutions which in general are expected to produce the greatest changes in protein properties will be those in which (a) a hydrophilic residue, e.g., seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g., leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine.

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FURTHER MODIFYING SEQUENCES OF THE INVENTION—MUTATION/ FORCED EVOLUTION

In addition to generating silent or conservative substitutions as noted, above, the present invention optionally includes methods of modifying the sequences of the Sequence Listing. In the methods, nucleic acid or protein modification methods are used to alter the given sequences to produce new sequences and/or to chemically or enzymatically modify given sequences to change the properties of the nucleic acids or proteins.

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Thus, in one embodiment, given nucleic acid sequences are modified, e.g., according to standard mutagenesis or artificial evolution methods to produce modified sequences. For example, Ausubel, *supra*, provides additional details on mutagenesis methods. Artificial forced evolution methods are described, e.g., by Stemmer (1994) Nature 370:389-391, and Stemmer (1994) Proc. Natl. Acad. Sci. USA 91:10747-10751. Many other mutation and evolution methods are also available and expected to be within the skill of the practitioner.

Similarly, chemical or enzymatic alteration of expressed nucleic acids and polypeptides can be performed by standard methods. For example, sequence can be modified by addition of lipids, sugars, peptides, organic or inorganic compounds, by the inclusion of modified nucleotides or amino acids, or the like. For example, protein modification techniques are illustrated in Ausubel, *supra*. Further details on chemical and enzymatic modifications can be found herein. These modification methods can be used to modify any given sequence, or to modify any sequence produced by the various mutation and artificial evolution modification methods noted herein.

Accordingly, the invention provides for modification of any given nucleic acid by mutation, evolution, chemical or enzymatic modification, or other available methods, as well as for the products produced by practicing such methods, e.g., using the sequences herein as a starting substrate for the various modification approaches.

For example, optimized coding sequence containing codons preferred by a particular prokaryotic or eukaryotic host can be used e.g., to increase the rate of translation or to produce recombinant RNA transcripts having desirable properties, such as a longer half-life, as compared with transcripts produced using a non-optimized sequence. Translation stop codons can also be modified to reflect host preference. For example, preferred stop codons for S. cerevisiae and mammals are TAA and TGA, respectively. The preferred stop codon for monocotyledonous plants is TGA, whereas insects and E. coli prefer to use TAA as the stop codon.

The polynucleotide sequences of the present invention can also be engineered in order to alter a coding sequence for a variety of reasons, including but not limited to, alterations which modify the sequence to facilitate cloning, processing and/or expression of

the gene product. For example, alterations are optionally introduced using techniques which are well known in the art, e.g., site-directed mutagenesis, to insert new restriction sites, to alter glycosylation patterns, to change codon preference, to introduce splice sites, etc.

Furthermore, a fragment or domain derived from any of the polypeptides of the invention can be combined with domains derived from other transcription factors or synthetic domains to modify the biological activity of a transcription factor. For instance, a DNA binding domain derived from a transcription factor of the invention can be combined with the activation domain of another transcription factor or with a synthetic activation domain. A transcription activation domain assists in initiating transcription from a DNA binding site. Examples include the transcription activation region of VP16 or GAL4 (Moore et al. (1998) Proc. Natl. Acad. Sci. USA 95: 376-381; and Aoyama et al. (1995) Plant Cell 7:1773-1785), peptides derived from bacterial sequences (Ma and Ptashne (1987) Cell 51; 113-119) and synthetic peptides (Giniger and Ptashne, (1987) Nature 330:670-672).

EXPRESSION AND MODIFICATION OF POLYPEPTIDES

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Typically, polynucleotide sequences of the invention are incorporated into recombinant DNA (or RNA) molecules that direct expression of polypeptides of the invention in appropriate host cells, transgenic plants, in vitro translation systems, or the like. Due to the inherent degeneracy of the genetic code, nucleic acid sequences which encode substantially the same or a functionally equivalent amino acid sequence can be substituted for any listed sequence to provide for cloning and expressing the relevant homologue.

Vectors, Promoters and Expression Systems

The present invention includes recombinant constructs comprising one or more of the nucleic acid sequences herein. The constructs typically comprise a vector, such as a plasmid, a cosmid, a phage, a virus (e.g., a plant virus), a bacterial artificial chromosome (BAC), a yeast artificial chromosome (YAC), or the like, into which a nucleic acid sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available.

General texts which describe molecular biological techniques useful herein, including the use and production of vectors, promoters and many other relevant topics, include Berger, Sambrook and Ausubel, *supra*. Any of the identified sequences can be incorporated into a cassette or vector, e.g., for expression in plants. A number of expression vectors suitable for stable transformation of plant cells or for the establishment of transgenic plants have been described including those described in Weissbach and Weissbach, (1989) Methods for Plant Molecular Biology, Academic Press, and Gelvin et al., (1990) Plant

Molecular Biology Manual, Kluwer Academic Publishers. Specific examples include those derived from a Ti plasmid of Agrobacterium tumefaciens, as well as those disclosed by Herrera-Estrella et al. (1983) Nature 303: 209, Bevan (1984) Nucl Acid Res. 12: 8711-8721, Klee (1985) Bio/Technology 3: 637-642, for dicotyledonous plants.

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Alternatively, non-Ti vectors can be used to transfer the DNA into monocotyledonous plants and cells by using free DNA delivery techniques. Such methods can involve, for example, the use of liposomes, electroporation, microprojectile bombardment, silicon carbide whiskers, and viruses. By using these methods transgenic plants such as wheat, rice (Christou (1991) Bio/Technology 9: 957-962) and corn (Gordon-10 Kamm (1990) Plant Cell 2: 603-618) can be produced. An immature embryo can also be a good target tissue for monocots for direct DNA delivery techniques by using the particle gun (Weeks et al. (1993) Plant Physiol 102: 1077-1084; Vasil (1993) Bio/Technology 10: 667-674; Wan and Lemeaux (1994) Plant Physiol 104: 37-48, and for Agrobacterium-mediated DNA transfer (Ishida et al. (1996) Nature Biotech 14: 745-750).

Typically, plant transformation vectors include one or more cloned plant coding sequence (genomic or cDNA) under the transcriptional control of 5' and 3' regulatory sequences and a dominant selectable marker. Such plant transformation vectors typically also contain a promoter (e.g., a regulatory region controlling inducible or constitutive, environmentally-or developmentally-regulated, or cell- or tissue-specific expression), a transcription initiation start site, an RNA processing signal (such as intron splice sites), a transcription termination site, and/or a polyadenylation signal.

Examples of constitutive plant promoters which can be useful for expressing the TF sequence include: the cauliflower mosaic virus (CaMV) 35S promoter, which confers constitutive, high-level expression in most plant tissues (see, e.g., Odel et al. (1985) Nature 313:810); the nopaline synthase promoter (An et al. (1988) Plant Physiol 88:547); and the octopine synthase promoter (Fromm et al. (1989) Plant Cell 1: 977).

A variety of plant gene promoters that regulate gene expression in response to environmental, hormonal, chemical, developmental signals, and in a tissue-active manner can be used for expression of a TF sequence in plants. Choice of a promoter is based largely on the phenotype of interest and is determined by such factors as tissue (e.g., seed, fruit, root, pollen, vascular tissue, flower, carpel, etc.), inducibility (e.g., in response to wounding, heat, cold, drought, light, pathogens, etc.), timing, developmental stage, and the like. Numerous known promoters have been characterized and can favorable be employed to promote expression of a polynucleotide of the invention in a transgenic plant or cell of interest. For example, tissue specific promoters include: seed-specific promoters (such as the napin, phaseolin or DC3 promoter described in US Pat. No. 5,773,697), fruit-specific promoters that are active during fruit ripening (such as the dru 1 promoter (US Pat. No. 5,783,393), or the

2A11 promoter (US Pat. No. 4,943,674) and the tomato polygalacturonase promoter (Bird et al. (1988) Plant Mol Biol 11:651), root-specific promoters, such as those disclosed in US Patent Nos. 5,618,988, 5,837,848 and 5,905,186, pollen-active promoters such as PTA29, PTA26 and PTA13 (US Pat. No. 5,792,929), promoters active in vascular tissue (Ringli and Keller (1998) Plant Mol Biol 37:977-988), flower-specific (Kaiser et al, (1995) Plant Mol Biol 28:231-243), pollen (Baerson et al. (1994) Plant Mol Biol 26:1947-1959), carpels (Ohl et al. (1990) Plant Cell 2:837-848), pollen and ovules (Baerson et al. (1993) Plant Mol Biol 22:255-267), auxin-inducible promoters (such as that described in van der Kop et al. (1999) Plant Mol Biol 39:979-990 or Baumann et al. (1999) Plant Cell 11:323-334), cytokinininducible promoter (Guevara-Garcia (1998) Plant Mol Biol 38:743-753), promoters responsive to gibberellin (Shi et al. (1998) Plant Mol Biol 38:1053-1060, Willmott et al. (1998) 38:817-825) and the like. Additional promoters are those that elicit expression in response to heat (Ainley et al. (1993) Plant Mol Biol 22: 13-23), light (e.g., the pea rbcS-3A promoter, Kuhlemeier et al. (1989) Plant Cell 1:471, and the maize rbcS promoter, Schaffner and Sheen (1991) Plant Cell 3: 997); wounding (e.g., wunI, Siebertz et al. (1989) Plant Cell 1: 961); pathogens (such as the PR-1 promoter described in Buchel et al. (1999) Plant Mol. Biol. 40:387-396, and the PDF1.2 promoter described in Manners et al. (1998) Plant Mol. Biol. 38:1071-80), and chemicals such as methyl jasmonate or salicylic acid (Gatz et al. (1997) Plant Mol Biol 48: 89-108). In addition, the timing of the expression can be controlled by using promoters such as those acting at senescence (An and Amazon (1995) Science 270: 1986-1988); or late seed development (Odell et al. (1994) Plant Physiol 106:447-458).

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Plant expression vectors can also include RNA processing signals that can be positioned within, upstream or downstream of the coding sequence. In addition, the expression vectors can include additional regulatory sequences from the 3'-untranslated region of plant genes, e.g., a 3' terminator region to increase mRNA stability of the mRNA, such as the PI-II terminator region of potato or the octopine or nopaline synthase 3' terminator regions.

Additional Expression Elements

Specific initiation signals can aid in efficient translation of coding sequences. These signals can include, e.g., the ATG initiation codon and adjacent sequences. In cases where a coding sequence, its initiation codon and upstream sequences are inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only coding sequence (e.g., a mature protein coding sequence), or a portion thereof, is inserted, exogenous transcriptional control signals including the ATG initiation codon can be separately provided. The initiation codon is provided in the correct reading frame to facilitate transcription. Exogenous transcriptional elements and initiation

codons can be of various origins, both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of enhancers appropriate to the cell system in use.

Expression Hosts

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The present invention also relates to host cells which are transduced with vectors of the invention, and the production of polypeptides of the invention (including fragments thereof) by recombinant techniques. Host cells are genetically engineered (i.e, nucleic acids are introduced, e.g., transduced, transformed or transfected) with the vectors of this invention, which may be, for example, a cloning vector or an expression vector comprising the relevant nucleic acids herein. The vector is optionally a plasmid, a viral particle, a phage, a naked nucleic acids, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants, or amplifying the relevant gene. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to those skilled in the art and in the references cited herein, including, Sambrook and Ausubel.

The host cell can be a eukaryotic cell, such as a yeast cell, or a plant cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Plant protoplasts are also suitable for some applications. For example, the DNA fragments are introduced into plant tissues, cultured plant cells or plant protoplasts by standard methods including electroporation (Fromm et al., (1985) Proc. Natl. Acad. Sci. USA 82, 5824, infection by viral vectors such as cauliflower mosaic virus (CaMV) (Hohn et al., (1982) Molecular Biology of Plant Tumors, (Academic Press, New York) pp. 549-560; US 4,407,956), high velocity ballistic penetration by small particles with the nucleic acid either within the matrix of small beads or particles, or on the surface (Klein et al., (1987) Nature 327, 70-73), use of pollen as vector (WO 85/01856), or use of Agrobacterium tumefaciens or A. rhizogenes carrying a T-DNA plasmid in which DNA fragments are cloned. The T-DNA plasmid is transmitted to plant cells upon infection by Agrobacterium tumefaciens, and a portion is stably integrated into the plant genome (Horsch et al. (1984) Science 233:496-498; Fraley et al. (1983) Proc. Natl. Acad. Sci. USA 80, 4803).

The cell can include a nucleic acid of the invention which encodes a polypeptide, wherein the cells expresses a polypeptide of the invention. The cell can also include vector sequences, or the like. Furthermore, cells and transgenic plants which include any polypeptide or nucleic acid above or throughout this specification, e.g., produced by transduction of a vector of the invention, are an additional feature of the invention.

For long-term, high-yield production of recombinant proteins, stable expression can be used. Host cells transformed with a nucleotide sequence encoding a polypeptide of the invention are optionally cultured under conditions suitable for the

expression and recovery of the encoded protein from cell culture. The protein or fragment thereof produced by a recombinant cell may be secreted, membrane-bound, or contained intracellularly, depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides encoding mature proteins of the invention can be designed with signal sequences which direct secretion of the mature polypeptides through a prokaryotic or eukaryotic cell membrane.

Modified Amino Acids

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Polypeptides of the invention may contain one or more modified amino acids. The presence of modified amino acids may be advantageous in, for example, increasing polypeptide half-life, reducing polypeptide antigenicity or toxicity, increasing polypeptide storage stability, or the like. Amino acid(s) are modified, for example, co-translationally or post-translationally during recombinant production or modified by synthetic or chemical means.

Non-limiting examples of a modified amino acid include incorporation or other use of acetylated amino acids, glycosylated amino acids, sulfated amino acids, prenylated (e.g., farnesylated, geranylgeranylated) amino acids, PEG modified (e.g., "PEGylated") amino acids, biotinylated amino acids, carboxylated amino acids, phosphorylated amino acids, etc. References adequate to guide one of skill in the modification of amino acids are replete throughout the literature.

20 IDENTIFICATION OF ADDITIONAL FACTORS

A transcription factor provided by the present invention can also be used to identify additional endogenous or exogenous molecules that can affect a phentoype or trait of interest. On the one hand, such molecules include organic (small or large molecules) and/or inorganic compounds that affect expression of (i.e., regulate) a particular transcription factor. Alternatively, such molecules include endogenous molecules that are acted upon either at a transcriptional level by a transcription factor of the invention to modify a phenotype as desired. For example, the transcription factors can be employed to identify one or more downstream gene with which is subject to a regulatory effect of the transcription factor. In one approach, a transcription factor or transcription factor homologue of the invention is expressed in a host cell, e.g, a transgenic plant cell, tissue or explant, and expression products, either RNA or protein, of likely or random targets are monitored, e.g., by hybridization to a microarray of nucleic acid probes corresponding to genes expressed in a tissue or cell type of interest, by two-dimensional gel electrophoresis of protein products, or by any other method known in the art for assessing expression of gene products at the level of RNA or protein. Alternatively, a transcription factor of the invention can be used to identify promoter sequences (i.e., binding sites) involved in the regulation of a downstream target. After

identifying a promoter sequence, interactions between the transcription factor and the promoter sequence can be modified by changing specific nucleotides in the promoter sequence or specific amino acids in the transcription factor that interact with the promoter sequence to alter a plant trait. Typically, transcription factor DNA binding sites are identified by gel shift assays. After identifying the promoter regions, the promoter region sequences can be employed in double-stranded DNA arrays to identify molecules that affect the interactions of the transcription factors with their promoters (Bulyk et al. (1999) Nature Biotechnology 17:573-577).

The identified transcription factors are also useful to identify proteins that modify the activity of the transcription factor. Such modification can occur by covalent modification, such as by phosphorylation, or by protein-protein (homo or-heteropolymer) interactions. Any method suitable for detecting protein-protein interactions can be employed. Among the methods that can be employed are co-immunoprecipitation, cross-linking and co-purification through gradients or chromatographic columns, and the two-hybrid yeast system.

The two-hybrid system detects protein interactions in vivo and is described in Chien, et al., (1991), Proc. Natl. Acad. Sci. USA 88, 9578-9582 and is commercially available from Clontech (Palo Alto, Calif.). In such a system, plasmids are constructed that encode two hybrid proteins: one consists of the DNA-binding domain of a transcription activator protein fused to the TF polypeptide and the other consists of the transcription activator protein's activation domain fused to an unknown protein that is encoded by a cDNA that has been recombined into the plasmid as part of a cDNA library. The DNA-binding domain fusion plasmid and the cDNA library are transformed into a strain of the yeast Saccharomyces cerevisiae that contains a reporter gene (e.g., lacZ) whose regulatory region contains the transcription activator's binding site. Either hybrid protein alone cannot activate transcription of the reporter gene. Interaction of the two hybrid proteins reconstitutes the functional activator protein and results in expression of the reporter gene, which is detected by an assay for the reporter gene product. Then, the library plasmids responsible for reporter gene expression are isolated and sequenced to identify the proteins encoded by the library plasmids. After identifying proteins that interact with the transcription factors, assays for compounds that interfere with the TF protein-protein interactions can be preformed.

IDENTIFICATION OF MODULATORS

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In addition to the intracellular molecules described above, extracellular molecules that alter activity or expression of a transcription factor, either directly or indirectly, can be identified. For example, the methods can entail first placing a candidate molecule in contact with a plant or plant cell. The molecule can be introduced by topical administration, such as spraying or soaking of a plant, and then the molecule's effect on the

expression or activity of the TF polypeptide or the expression of the polynucleotide monitored. Changes in the expression of the TF polypeptide can be monitored by use of polyclonal or monoclonal antibodies, gel electrophoresis or the like. Changes in the expression of the corresponding polynucleotide sequence can be detected by use of microarrays, Northerns, quantitative PCR, or any other technique for monitoring changes in mRNA expression. These techniques are exemplified in Ausubel et al. (eds) <u>Current Protocols in Molecular Biology</u>, John Wiley & Sons (1998). Such changes in the expression levels can be correlated with modified plant traits and thus identified molecules can be useful for soaking or spraying on fruit, vegetable and grain crops to modify traits in plants.

Essentially any available composition can be tested for modulatory activity of expression or activity of any nucleic acid or polypeptide herein. Thus, available libraries of compounds such as chemicals, polypeptides, nucleic acids and the like can be tested for modulatory activity. Often, potential modulator compounds can be dissolved in aqueous or organic (e.g., DMSO-based) solutions for easy delivery to the cell or plant of interest in which the activity of the modulator is to be tested. Optionally, the assays are designed to screen large modulator composition libraries by automating the assay steps and providing compounds from any convenient source to assays, which are typically run in parallel (e.g., in microtiter formats on microtiter plates in robotic assays).

In one embodiment, high throughput screening methods involve providing a combinatorial library containing a large number of potential compounds (potential modulator compounds). Such "combinatorial chemical libraries" are then screened in one or more assays, as described herein, to identify those library members (particular chemical species or subclasses) that display a desired characteristic activity. The compounds thus identified can serve as target compounds.

A combinatorial chemical library can be, e.g., a collection of diverse chemical compounds generated by chemical synthesis or biological synthesis. For example, a combinatorial chemical library such as a polypeptide library is formed by combining a set of chemical building blocks (e.g., in one example, amino acids) in every possible way for a given compound length (i.e., the number of amino acids in a polypeptide compound of a set length). Exemplary libraries include peptide libraries, nucleic acid libraries, antibody libraries (see, e.g., Vaughn et al. (1996) Nature Biotechnology, 14(3):309-314 and PCT/US96/10287), carbohydrate libraries (see, e.g., Liang et al. Science (1996) 274:1520-1522 and U.S. Patent 5,593,853), peptide nucleic acid libraries (see, e.g., U.S. Patent 5,539,083), and small organic molecule libraries (see, e.g., benzodiazepines, Baum C&EN Jan 18, page 33 (1993); isoprenoids, U.S. Patent 5,569,588; thiazolidinones and metathiazanones, U.S. Patent 5,549,974; pyrrolidines, U.S. Patents 5,525,735 and 5,519,134; morpholino compounds, U.S. Patent 5,506,337) and the like.

Preparation and screening of combinatorial or other libraries is well known to those of skill in the art. Such combinatorial chemical libraries include, but are not limited to, peptide libraries (see, e.g., U.S. Patent 5,010,175, Furka, Int. J. Pept. Prot. Res. 37:487-493 (1991) and Houghton et al. Nature 354:84-88 (1991)). Other chemistries for generating chemical diversity libraries can also be used.

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In addition, as noted, compound screening equipment for high-throughput screening is generally available, e.g., using any of a number of well known robotic systems that have also been developed for solution phase chemistries useful in assay systems. These systems include automated workstations including an automated synthesis apparatus and robotic systems utilizing robotic arms. Any of the above devices are suitable for use with the present invention, e.g., for high-throughput screening of potential modulators. The nature and implementation of modifications to these devices (if any) so that they can operate as discussed herein will be apparent to persons skilled in the relevant art.

Indeed, entire high throughput screening systems are commercially available. These systems typically automate entire procedures including all sample and reagent pipetting, liquid dispensing, timed incubations, and final readings of the microplate in detector(s) appropriate for the assay. These configurable systems provide high throughput and rapid start up as well as a high degree of flexibility and customization. Similarly, microfluidic implementations of screening are also commercially available.

The manufacturers of such systems provide detailed protocols the various high throughput. Thus, for example, Zymark Corp. provides technical bulletins describing screening systems for detecting the modulation of gene transcription, ligand binding, and the like. The integrated systems herein, in addition to providing for sequence alignment and, optionally, synthesis of relevant nucleic acids, can include such screening apparatus to identify modulators that have an effect on one or more polynucleotides or polypeptides according to the present invention.

In some assays it is desirable to have positive controls to ensure that the components of the assays are working properly. At least two types of positive controls are appropriate. That is, known transcriptional activators or inhibitors can be incubated with cells/plants/ etc. in one sample of the assay, and the resulting increase/decrease in transcription can be detected by measuring the resulting increase in RNA/ protein expression, etc., according to the methods herein. It will be appreciated that modulators can also be combined with transcriptional activators or inhibitors to find modulators which inhibit transcriptional activation or transcriptional repression. Either expression of the nucleic acids and proteins herein or any additional nucleic acids or proteins activated by the nucleic acids or proteins herein, or both, can be monitored.

In an embodiment, the invention provides a method for identifying compositions that modulate the activity or expression of a polynucleotide or polypeptide of the invention. For example, a test compound, whether a small or large molecule, is placed in contact with a cell, plant (or plant tissue or explant), or composition comprising the polynucleotide or polypeptide of interest and a resulting effect on the cell, plant, (or tissue or explant) or composition is evaluated by monitoring, either directly or indirectly, one or more of: expression level of the polynucleotide or polypeptide, activity (or modulation of the activity) of the polynucleotide or polypeptide. In some cases, an alteration in a plant phenotype can be detected following contact of a plant (or plant cell, or tissue or explant) with the putative modulator, e.g., by modulation of expression or activity of a polynucleotide or polypeptide of the invention.

SUBSEQUENCES

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Also contemplated are uses of polynucleotides, also referred to herein as oligonucleotides, typically having at least 12 bases, preferably at least 15, more preferably at least 20, 30, or 50 bases, which hybridize under at least highly stringent (or ultra-high stringent or ultra-ultra- high stringent conditions) conditions to a polynucleotide sequence described above. The polynucleotides may be used as probes, primers, sense and antisense agents, and the like, according to methods as noted *supra*.

Subsequences of the polynucleotides of the invention, including polynucleotide fragments and oligonucleotides are useful as nucleic acid probes and primers. An oligonucleotide suitable for use as a probe or primer is at least about 15 nucleotides in length, more often at least about 18 nucleotides, often at least about 21 nucleotides, frequently at least about 30 nucleotides, or about 40 nucleotides, or more in length. A nucleic acid probe is useful in hybridization protocols, e.g., to identify additional polypeptide homologues of the invention, including protocols for microarray experiments. Primers can be annealed to a complementary target DNA strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand, and then extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR) or other nucleic-acid amplification methods. See Sambrook and Ausubel, *supra*.

In addition, the invention includes an isolated or recombinant polypeptide including a subsequence of at least about 15 contiguous amino acids encoded by the recombinant or isolated polynucleotides of the invention. For example, such polypeptides, or domains or fragments thereof, can be used as immunogens, e.g., to produce antibodies specific for the polypeptide sequence, or as probes for detecting a sequence of interest. A

subsequence can range in size from about 15 amino acids in length up to and including the full length of the polypeptide.

PRODUCTION OF TRANSGENIC PLANTS

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Modification of Traits

The polynucleotides of the invention are favorably employed to produce transgenic plants with various traits, or characteristics, that have been modified in a desirable manner, e.g., to improve the pathogen resistance of a plant. For example, alteration of expression levels or patterns (e.g., spatial or temporal expression patterns) of one or more of the transcription factors (or transcription factor homologues) of the invention, as compared with the levels of the same protein found in a wild type plant, can be used to modify a plant's traits. An illustrative example of trait modification, improved pathogen tolerance, by altering expression levels of a particular transcription factor is described further in the Examples and the Sequence Listing.

Antisense and Cosuppression Approaches

In addition to expression of the nucleic acids of the invention as gene replacement or plant phenotype modification nucleic acids, the nucleic acids are also useful for sense and anti-sense suppression of expression, e.g., to down-regulate expression of a nucleic acid of the invention, e.g., as a further mechanism for modulating plant phenotype. That is, the nucleic acids of the invention, or subsequences or anti-sense sequences thereof, can be used to block expression of naturally occurring homologous nucleic acids. A variety of sense and anti-sense technologies are known in the art, e.g., as set forth in Lichtenstein and Nellen (1997) Antisense Technology: A Practical Approach IRL Press at Oxford University, Oxford, England. In general, sense or anti-sense sequences are introduced into a cell, where they are optionally amplified, e.g., by transcription. Such sequences include both simple oligonucleotide sequences and catalytic sequences such as ribozymes.

For example, a reduction or elimination of expression (i.e., a "knock-out") of a transcription factor or transcription factor homologue polypeptide in a transgenic plant, e.g., to modify a plant trait, can be obtained by introducing an antisense construct corresponding to the polypeptide of interest as a cDNA. For antisense suppression, the transcription factor or homologue cDNA is arranged in reverse orientation (with respect to the coding sequence) relative to the promoter sequence in the expression vector. The introduced sequence need not be the full length cDNA or gene, and need not be identical to the cDNA or gene found in the plant type to be transformed. Typically, the antisense sequence need only be capable of hybridizing to the target gene or RNA of interest. Thus, where the introduced sequence is of shorter length, a higher degree of homology to the endogenous transcription factor sequence will be needed for effective antisense suppression. While antisense sequences of various

lengths can be utilized, preferably, the introduced antisense sequence in the vector will be at least 30 nucleotides in length, and improved antisense suppression will typically be observed as the length of the antisense sequence increases. Preferably, the length of the antisense sequence in the vector will be greater than 100 nucleotides. Transcription of an antisense construct as described results in the production of RNA molecules that are the reverse complement of mRNA molecules transcribed from the endogenous transcription factor gene in the plant cell.

Suppression of endogenous transcription factor gene expression can also be achieved using a ribozyme. Ribozymes are RNA molecules that possess highly specific endoribonuclease activity. The production and use of ribozymes are disclosed in U.S. Patent No. 4,987,071 and U.S. Patent No. 5,543,508. Synthetic ribozyme sequences including antisense RNAs can be used to confer RNA cleaving activity on the antisense RNA, such that endogenous mRNA molecules that hybridize to the antisense RNA are cleaved, which in turn leads to an enhanced antisense inhibition of endogenous gene expression.

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Vectors in which RNA encoded by a transcription factor or transcription factor homologue cDNA is over-expressed can also be used to obtain co-suppression of a corresponding endogenous gene, e.g., in the manner described in U.S. Patent No. 5,231,020 to Jorgensen. Such co-suppression (also termed sense suppression) does not require that the entire transcription factor cDNA be introduced into the plant cells, nor does it require that the introduced sequence be exactly identical to the endogenous transcription factor gene of interest. However, as with antisense suppression, the suppressive efficiency will be enhanced as specificity of hybridization is increased, e.g., as the introduced sequence is lengthened, and/or as the sequence similarity between the introduced sequence and the endogenous transcription factor gene is increased.

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Vectors expressing an untranslatable form of the transcription factor mRNA, e.g., sequences comprising one or more stop codon, or nonsense mutation) can also be used to suppress expression of an endogenous transcription factor, thereby reducing or eliminating it's activity and modifying one or more traits. Methods for producing such constructs are described in U.S. Patent No. 5,583,021. Preferably, such constructs are made by introducing a premature stop codon into the transcription factor gene. Alternatively, a plant trait can be modified by gene silencing using double-strand RNA (Sharp (1999) Genes and Development 13: 139-141).

Another method for abolishing the expression of a gene is by insertion mutagenesis using the T-DNA of *Agrobacterium tumefaciens*. After generating the insertion mutants, the mutants can be screened to identify those containing the insertion in a transcription factor or transcription factor homologue gene. Plants containing a single

transgene insertion event at the desired gene can be crossed to generate homozygous plants for the mutation (Koncz et al. (1992) Methods in Arabidopsis Research, World Scientific).

Alternatively, a plant phenotype can be altered by eliminating an endogenous gene, such as a transcription factor or transcription factor homologue, e.g., by homologous recombination (Kempin et al. (1997) <u>Nature</u> 389:802).

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A plant trait can also be modified by using the cre-lox system (for example, as described in US Paent No. 5,658,772). A plant genome can be modified to include first and second lox sites that are then contacted with a Cre recombinase. If the lox sites are in the same orientation, the intervening DNA sequence between the two sites is excised. If the lox sites are in the opposite orientation, the intervening sequence is inverted.

The polynucleotides and polypeptides of this invention can also be expressed in a plant in the absence of an expression cassette by manipulating the activity or expression level of the endogenous gene by other means. For example, by ectopically expressing a gene by T-DNA activation tagging (Ichikawa et al. (1997) Nature 390 698-701; Kakimoto et al. (1996) Science 274: 982-985). This method entails transforming a plant with a gene tag containing multiple transcriptional enhancers and once the tag has inserted into the genome, expression of a flanking gene coding sequence becomes deregulated. In another example, the transcriptional machinery in a plant can be modified so as to increase transcription levels of a polynucleotide of the invention (See, e.g., PCT Publications WO 96/06166 and WO 98/53057 which describe the modification of the DNA binding specificity of zinc finger proteins by changing particular amino acids in the DNA binding motif).

The transgenic plant can also include the machinery necessary for expressing or altering the activity of a polypeptide encoded by an endogenous gene, for example by altering the phosphorylation state of the polypeptide to maintain it in an activated state.

Transgenic plants (or plant cells, or plant explants, or plant tissues) incorporating the polynucleotides of the invention and/or expressing the polypeptides of the invention can be produced by a variety of well established techniques as described above. Following construction of a vector, most typically an expression cassette, including a polynucleotide, e.g., encoding a transcription factor or transcription factor homologue, of the invention, standard techniques can be used to introduce the polynucleotide into a plant, a plant cell, a plant explant or a plant tissue of interest. Optionally, the plant cell, explant or tissue can be regenerated to produce a transgenic plant.

The plant can be any higher plant, including gymnosperms, monocotyledonous and dicotyledenous plants. Suitable protocols are available for *Leguminosae* (alfalfa, soybean, clover, etc.), *Umbelliferae* (carrot, celery, parsnip), *Cruciferae* (cabbage, radish, rapeseed, broccoli, etc.), *Curcurbitaceae* (melons and cucumber), *Gramineae* (wheat, corn, rice, barley, millet, etc.), *Solanaceae* (potato, tomato, tobacco,

peppers, etc.), and various other crops. See protocols described in Ammirato et al. (1984) Handbook of Plant Cell Culture -Crop Species. Macmillan Publ. Co. Shimamoto et al. (1989) Nature 338:274-276; Fromm et al. (1990) Bio/Technology 8:833-839; and Vasil et al. (1990) Bio/Technology 8:429-434.

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Transformation and regeneration of both monocotyledonous and dicotyledonous plant cells is now routine, and the selection of the most appropriate transformation technique will be determined by the practitioner. The choice of method will vary with the type of plant to be transformed; those skilled in the art will recognize the suitability of particular methods for given plant types. Suitable methods can include, but are not limited to: electroporation of plant protoplasts; liposome-mediated transformation; polyethylene glycol (PEG) mediated transformation; transformation using viruses; microinjection of plant cells; micro-projectile bombardment of plant cells; vacuum infiltration; and Agrobacterium tumeficiens mediated transformation. Transformation means introducing a nucleotide sequence in a plant in a manner to cause stable or transient expression of the sequence.

Successful examples of the modification of plant characteristics by transformation with cloned sequences which serve to illustrate the current knowledge in this field of technology, and which are herein incorporated by reference, include: U.S. Patent Nos. 5,571,706; 5,677,175; 5,510,471; 5,750,386; 5,597,945; 5,589,615; 5,750,871; 5,268,526; 5,780,708; 5,538,880; 5,773,269; 5,736,369 and 5,610,042.

Following transformation, plants are preferably selected using a dominant selectable marker incorporated into the transformation vector. Typically, such a marker will confer antibiotic or herbicide resistance on the transformed plants, and selection of transformants can be accomplished by exposing the plants to appropriate concentrations of the antibiotic or herbicide.

After transformed plants are selected and grown to maturity, those plants showing a modified trait are identified. The modified trait can be any of those traits described above. Additionally, to confirm that the modified trait is due to changes in expression levels or activity of the polypeptide or polynucleotide of the invention can be determined by analyzing mRNA expression using Northern blots, RT-PCR or microarrays, or protein expression using immunoblots or Western blots or gel shift assays.

INTEGRATED SYSTEMS—SEQUENCE IDENTITY

Additionally, the present invention may be an integrated system, computer or computer readable medium that comprises an instruction set for determining the identity of one or more sequences in a database. In addition, the instruction set can be used to generate or identify sequences that meet any specified criteria. Furthermore, the instruction set may

be used to associate or link certain functional benefits, such improved pathogen tolerance, with one or more identified sequence.

For example, the instruction set can include, e.g., a sequence comparison or other alignment program, e.g., an available program such as, for example, the Wisconsin Package Version 10.0, such as BLAST, FASTA, PILEUP, FINDPATTERNS or the like (GCG, Madision, WI). Public sequence databases such as GenBank, EMBL, Swiss-Prot and PIR or private sequence databases such as PhytoSeq (Incyte Pharmaceuticals, Palo Alto, CA) can be searched.

Alignment of sequences for comparison can be conducted by the local homology algorithm of Smith and Waterman (1981) Adv. Appl. Math. 2:482, by the homology alignment algorithm of Needleman and Wunsch (1970) J. Mol. Biol. 48:443, by the search for similarity method of Pearson and Lipman (1988) Proc. Natl. Acad. Sci. U.S.A. 85: 2444, by computerized implementations of these algorithms. After alignment, sequence comparisons between two (or more) polynucleotides or polypeptides are typically performed by comparing sequences of the two sequences over a comparison window to identify and compare local regions of sequence similarity. The comparison window can be a segment of at least about 20 contiguous positions, usually about 50 to about 200, more usually about 100 to about 150 contiguous positions. A description of the method is provided in Ausubel et al., supra.

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A variety of methods of determining sequence relationships can be used, including manual alignment and computer assisted sequence alignment and analysis. This later approach is a preferred approach in the present invention, due to the increased throughput afforded by computer assisted methods. As noted above, a variety of computer programs for performing sequence alignment are available, or can be produced by one of skill.

One example algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul et al. J. Mol. Biol 215:403-410 (1990). Software for performing BLAST analyses is publicly available, e.g., through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul et al., supra). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters

M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, a cutoff of 100, M=5, N=-4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff (1989) Proc. Natl. Acad. Sci. USA 89:10915).

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In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin & Altschul (1993) Proc. Natl. Acad. Sci. USA 90:5873-5787). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence (and, therefore, in this context, homologous) if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.1, or less than about 0.01, and or even less than about 0.001. An additional example of a useful sequence alignment algorithm is PILEUP. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments. The program can align, e.g., up to 300 sequences of a maximum length of 5,000 letters.

The integrated system, or computer typically includes a user input interface allowing a user to selectively view one or more sequence records corresponding to the one or more character strings, as well as an instruction set which aligns the one or more character strings with each other or with an additional character string to identify one or more region of sequence similarity. The system may include a link of one or more character strings with a particular phenotype or gene function. Typically, the system includes a user readable output element which displays an alignment produced by the alignment instruction set.

The methods of this invention can be implemented in a localized or distributed computing environment. In a distributed environment, the methods may implemented on a single computer comprising multiple processors or on a multiplicity of computers. The computers can be linked, e.g. through a common bus, but more preferably the computer(s) are nodes on a network. The network can be a generalized or a dedicated local or

wide-area network and, in certain preferred embodiments, the computers may be components of an intra-net or an internet.

Thus, the invention provides methods for identifying a sequence similar or homologous to one or more polynucleotides as noted herein, or one or more target polypeptides encoded by the polynucleotides, or otherwise noted herein and may include linking or associating a given plant phenotype or gene function with a sequence. In the methods, a sequence database is provided (locally or across an inter or intra net) and a query is made against the sequence database using the relevant sequences herein and associated plant phenotypes or gene functions.

Any sequence herein can be entered into the database, before or after querying the database. This provides for both expansion of the database and, if done before the querying step, for insertion of control sequences into the database. The control sequences can be detected by the query to ensure the general integrity of both the database and the query. As noted, the query can be performed using a web browser based interface. For example, the database can be a centralized public database such as those noted herein, and the querying can be done from a remote terminal or computer across an internet or intranet.

EXAMPLES

The following examples are intended to illustrate but not limit the present invention.

20 EXAMPLE I, FULL LENGTH GENE IDENTIFICATION AND CLONING

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Putative transcription factor sequences (genomic or ESTs) related to known transcription factors were identified in the *Arabidopsis thaliana* GenBank database using the tblastn sequence analysis program using default parameters and a P-value cutoff threshold of -4 or -5 or lower, depending on the length of the query sequence. Putative transcription factor sequence hits were then screened to identify those containing particular sequence strings. If the sequence hits contained such sequence strings, the sequences were confirmed as transcription factors.

Alternatively, Arabidopsis thaliana cDNA libraries derived from different tissues or treatments, or genomic libraries were screened to identify novel members of a transcription family using a low stringency hybridization approach. Probes were synthesized using gene specific primers in a standard PCR reaction (annealing temperature 60° C) and labeled with ³²P dCTP using the High Prime DNA Labeling Kit (Boehringer Mannheim). Purified radiolabelled probes were added to filters immersed in Church hybridization medium (0.5 M NaPO₄ pH 7.0, 7% SDS, 1 % w/v bovine serum albumin) and hybridized overnight at 60 °C with shaking. Filters were washed two times for 45 to 60 minutes with 1xSCC, 1% SDS at 60° C.

To identify additional sequence 5' or 3' of a partial cDNA sequence in a cDNA library, 5' and 3' rapid amplification of cDNA ends (RACE) was performed using the MarathonTM cDNA amplification kit (Clontech, Palo Alto, CA). Generally, the method entailed first isolating poly(A) mRNA, performing first and second strand cDNA synthesis to generate double stranded cDNA, blunting cDNA ends, followed by ligation of the MarathonTM Adaptor to the cDNA to form a library of adaptor-ligated ds cDNA.

Gene-specific primers were designed to be used along with adaptor specific primers for both 5' and 3' RACE reactions. Nested primers, rather than single primers, were used to increase PCR specificity. Using 5' and 3' RACE reactions, 5' and 3' RACE fragments were obtained, sequenced and cloned. The process can be repeated until 5' and 3' ends of the full-length gene were identified. Then the full-length cDNA was generated by PCR using primers specific to 5' and 3' ends of the gene by end-to-end PCR.

EXAMPLE II. CONSTRUCTION OF EXPRESSION VECTORS

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The sequence was amplified from a genomic or cDNA library using primers 15 specific to sequences upstream and downstream of the coding region. The expression vector was pMEN20 or pMEN65, which are both derived from pMON316 (Sanders et al, (1987) Nucleic Acids Research 15:1543-58) and contain the CaMV 35S promoter to express transgenes. To clone the sequence into the vector, both pMEN20 and the amplified DNA fragment were digested separately with SalI and NotI restriction enzymes at 37° C for 2 hours. 20 The digestion products were subject to electrophoresis in a 0.8% agarose gel and visualized by ethidium bromide staining. The DNA fragments containing the sequence and the linearized plasmid were excised and purified by using a Qiaquick gel extraction kit (Qiagen, CA). The fragments of interest were ligated at a ratio of 3:1 (vector to insert). Ligation reactions using T4 DNA ligase (New England Biolabs, MA) were carried out at 16° C for 16 25 hours. The ligated DNAs were transformed into competent cells of the E. coli strain DH5alpha by using the heat shock method. The transformations were plated on LB plates containing 50 mg/l kanamycin (Sigma).

Individual colonies were grown overnight in five milliliters of LB broth containing 50 mg/l kanamycin at 37° C. Plasmid DNA was purified by using Qiaquick Mini Prep kits (Qiagen, CA).

EXAMPLE III. TRANSFORMATION OF AGROBACTERIUM WITH THE EXPRESSION VECTOR

After the plasmid vector containing the gene was constructed, the vector was used to transform Agrobacterium tumefaciens cells expressing the gene products. The stock of Agrobacterium tumefaciens cells for transformation were made as described by Nagel et al. (1990) FEMS Microbiol Letts. 67: 325-328. Agrobacterium strain ABI was grown in 250 ml

LB medium (Sigma) overnight at 28°C with shaking until an absorbance (A_{600}) of 0.5 – 1.0 was reached. Cells were harvested by centrifugation at 4,000 x g for 15 min at 4° C. Cells were then resuspended in 250 μ l chilled buffer (1 mM HEPES, pH adjusted to 7.0 with KOH). Cells were centrifuged again as described above and resuspended in 125 μ l chilled buffer. Cells were then centrifuged and resuspended two more times in the same HEPES buffer as described above at a volume of 100 μ l and 750 μ l, respectively. Resuspended cells were then distributed into 40 μ l aliquots, quickly frozen in liquid nitrogen, and stored at -80° C.

Agrobacterium cells were transformed with plasmids prepared as described above following the protocol described by Nagel et al. For each DNA construct to be transformed, 50 – 100 ng DNA (generally resuspended in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0) was mixed with 40 μl of Agrobacterium cells. The DNA/cell mixture was then transferred to a chilled cuvette with a 2mm electrode gap and subject to a 2.5 kV charge dissipated at 25 μF and 200 μF using a Gene Pulser II apparatus (Bio-Rad). After electroporation, cells were immediately resuspended in 1.0 ml LB and allowed to recover without antibiotic selection for 2 – 4 hours at 28° C in a shaking incubator. After recovery, cells were plated onto selective medium of LB broth containing 100 μg/ml spectinomycin (Sigma) and incubated for 24-48 hours at 28° C. Single colonies were then picked and inoculated in fresh medium. The presence of the plasmid construct was verified by PCR amplification and sequence analysis.

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EXAMPLE IV. TRANSFORMATION OF ARABIDOPSIS PLANTS WITH AGROBACTERIUM TUMEFACIENS WITH EXPRESSION VECTOR

After transformation of Agrobacterium tumefaciens with plasmid vectors containing the gene, single Agrobacterium colonies were identified, propagated, and used to transform Arabidopsis plants. Briefly, 500 ml cultures of LB medium containing 50 mg/l kanamycin were inoculated with the colonies and grown at 28° C with shaking for 2 days until an absorbance (A_{600}) of > 2.0 is reached. Cells were then harvested by centrifugation at 4,000 x g for 10 min, and resuspended in infiltration medium (1/2 X Murashige and Skoog salts (Sigma), 1 X Gamborg's B-5 vitamins (Sigma), 5.0% (w/v) sucrose (Sigma), 0.044 μ M benzylamino purine (Sigma), 200 μ l/L Silwet L-77 (Lehle Seeds) until an absorbance (A_{600}) of 0.8 was reached.

Prior to transformation, Arabidopsis thaliana seeds (ecotype Columbia) were sown at a density of ~10 plants per 4" pot onto Pro-Mix BX potting medium (Hummert International) covered with fiberglass mesh (18 mm X 16 mm). Plants were grown under continuous illumination (50-75 μ E/m²/sec) at 22-23° C with 65-70% relative humidity. After about 4 weeks, primary inflorescence stems (bolts) are cut off to encourage growth of

multiple secondary bolts. After flowering of the mature secondary bolts, plants were prepared for transformation by removal of all siliques and opened flowers.

The pots were then immersed upside down in the mixture of Agrobacterium infiltration medium as described above for 30 sec, and placed on their sides to allow draining into a 1' x 2' flat surface covered with plastic wrap. After 24 h, the plastic wrap was removed and pots are turned upright. The immersion procedure was repeated one week later, for a total of two immersions per pot. Seeds were then collected from each transformation pot and analyzed following the protocol described below.

EXAMPLE V. IDENTIFICATION OF ARABIDOPSIS PRIMARY TRANSFORMANTS

Seeds collected from the transformation pots were sterilized essentially as follows. Seeds were dispersed into in a solution containing 0.1% (v/v) Triton X-100 (Sigma) and sterile H₂O and washed by shaking the suspension for 20 min. The wash solution was then drained and replaced with fresh wash solution to wash the seeds for 20 min with shaking. After removal of the second wash solution, a solution containing 0.1% (v/v) Triton X-100 and 70% ethanol (Equistar) was added to the seeds and the suspension was shaken for 5 min. After removal of the ethanol/detergent solution, a solution containing 0.1% (v/v) Triton X-100 and 30% (v/v) bleach (Clorox) was added to the seeds, and the suspension was shaken for 10 min. After removal of the bleach/detergent solution, seeds were then washed five times in sterile distilled H₂O. The seeds were stored in the last wash water at 4° C for 2 days in the dark before being plated onto antibiotic selection medium (1 X Murashige and Skoog salts (pH adjusted to 5.7 with 1M KOH), 1 X Gamborg's B-5 vitamins, 0.9% phytagar (Life Technologies), and 50 mg/l kanamycin). Seeds were germinated under continuous illumination (50-75 µE/m²/sec) at 22-23° C. After 7-10 days of growth under these conditions, kanamycin resistant primary transformants (T₁ generation) were visible and obtained. These seedlings were transferred first to fresh selection plates where the seedlings continued to grow for 3-5 more days, and then to soil (Pro-Mix BX potting medium).

Primary transformants were crossed and progeny seeds (T₂) collected; kanamycin resistant seedlings were selected and analyzed. The expression levels of the recombinant polynucleotides in the transformants varies from about a 5% expression level increase to a least a 100% expression level increase. Similar observations are made with respect to polypeptide level expression.

EXAMPLE VI. IDENTIFICATION OF ARABIDOPSIS PLANTS WITH TRANSCRIPTION FACTOR GENE KNOCKOUTS

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The screening of insertion mutagenized Arabidopsis collections for null mutants in a known target gene was essentially as described in Krysan et al (1999) Plant Cell 11:2283-2290. Briefly, gene-specific primers, nested by 5-250 bases to each other, were designed from the 5' and 3' regions of a known target gene. Similarly, nested sets of primers were also created specific to each of the T-DNA or transposon ends (the "right" and "left" borders). All possible combinations of gene specific and T-DNA/transposon primers were used to detect by PCR an insertion event within or close to the target gene. The amplified DNA fragments were then sequenced which allows the precise determination of the T-DNA/transposon insertion point relative to the target gene. Insertion events within the coding or intervening sequence of the genes were deconvoluted from a pool comprising a plurality of insertion events to a single unique mutant plant for functional characterization. The method is described in more detail in Yu and Adam, US Application Serial No. 09/177,733 filed October 23, 1998.

15 EXAMPLE VII. IDENTIFICATION OF PATHOGEN INDUCED GENES

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In some instances, expression patterns of the pathogen induced genes (such as defense genes) was monitored by microarray experiments. cDNAs were generated by PCR and resuspended at a final concentration of ~ 100 ng/ul in 3X SSC or 150mM Na-phosphate (Eisen and Brown (1999) *Meth. in Enzymol.* 303:179-205). The cDNAs were spotted on microscope glass slides coated with polylysine. The prepared cDNAs were aliquoted into 384 well plates and spotted on the slides using an x-y-z gantry (OmniGrid) purchased from GeneMachines (Menlo Park, CA) outfitted with quill type pins purchased from Telechem International (Sunnyvale, CA). After spotting, the arrays were cured for a minimum of one week at room temperature, rehydrated and blocked following the protocol recommended by Eisen and Brown (1999).

Sample total RNA (10 ug) samples were labeled using fluorescent Cy3 and Cy5 dyes. Labeled samples were resuspended in 4X SSC/0.03% SDS/4 ug salmon sperm DNA/2 ug tRNA/ 50mM Na-pyrophosphate, heated for 95°C for 2.5 minutes, spun down and placed on the array. The array was then covered with a glass coverslip and placed in a sealed chamber. The chamber was then kept in a water bath at 62°C overnight. The arrays were washed as described in Eisen and Brown (1999) and scanned on a General Scanning 3000 laser scanner. The resulting files are subsequently quantified using Imagene a software purchased from BioDiscovery (Los Angeles, CA).

EXAMPLE VIII. IDENTIFICATION OF PATHOGEN TOLERANCE PHENOTYPE IN OVEREXPRESSOR OR GENE KNOCKOUT PLANTS

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Experiments were performed to identify those transformants or knockouts that exhibited an improved pathogen tolerance. For such studies, the transformants were exposed to biotropic fungal pathogens, such as Erisyphe orontii, and necrotropic fungal pathogens, such as Fusarium oxysporum. Fusarium oxysporum isolates cause vascular wilts and damping off of various annual vegetables, perennials and weeds (Mauch-Mani and Slusarenko (1994) Molecular Plant-Microbe Interactions 7: 378-383). For Fusarium oxysporum experiments, plants grown on petri dishes were sprayed with a fresh spore suspension of F. oxysporum. The spore suspension was prepared as follows: A plug of fungal hyphae from a plate culture was placed on a fresh potato dextrose agar plate and allowed to spread for one week. 5 ml sterile water was then added to the plate, swirled, and pipetted into 50 ml Armstrong Fusarium medium. Spores were grown overnight in Fusarium medium and then sprayed onto plants using a Preval paint sprayer. Plant tissue was harvested and frozen in liquid nitrogen 48 hours post infection.

Erysiphe orontii is a causal agent of powdery mildew. For Erysiphe orontii experiments, plants were grown approximately 4 weeks in a greenhouse under 12 hour light (20 C, ~30% relative humidity (rh)). Individual leaves were infected with E. orontii spores from infected plants using a camel's hair brush, and the plants were transferred to a Percival growth chamber (20 C, 80% rh.). Plant tissue was harvested and frozen in liquid nitrogen 7 days post infection.

Botrytis cinerea is a necrotrophic pathogen. Botrytis cinerea was grown on potato dextrose agar in the light. A spore culture was made by spreading 10 ml of sterile water on the fungus plate, swirling and transferring spores to 10 ml of sterile water. The spore inoculum (approx. 105 spores/ml) was used to spray 10 day-old seedlings grown under sterile conditions on MS (-sucrose) media. Symptoms were evaluated every day up to approximately 1 week.

Infection with bacterial pathogens Pseudomonas syringae pv maculicola strain 4326 and pv maculicola strain 4326 was performed by hand inoculation at two doses. Two inoculation doses allows the differentiation between plants with enhanced susceptibility and plants with enhanced resistance to the pathogen. Plants were grown for 3 weeks in the greenhouse, then transferred to the growth chamber for the remainder of their growth. Psm ES4326 was hand inoculated with 1 ml syringe on 3 fully-expanded leaves per plant (4 1/2 wk old), using at least 9 plants per overexpressing line at two inoculation doses, OD=0.005 and OD=0.0005. Disease scoring occurred at day 3 post-inoculation with pictures of the plants and leaves taken in parallel.

Table 3 shows the phenotypes observed for particular overexpressor or knockout plants and provides the SEQ ID No., the internal reference code (GID), whether a knockout or overexpressor plant was analyzed and the observed phenotype.

Table 3

SEQ ID No.	GID	Knockout (KO) or overexpressor (OE)	Phenotype
1	G188	КО	Increased susceptibility to Fusarium
3	G616	OE	Increased tolerance to Erysiphe
5	G19	OE	Increased tolerance to Erysiphe
7.	G261	OE	Increased susceptibility to Botrytis
9	G28	OE	Increased resistance to Erysiphe
11	G869	OE	Increased susceptibility to Fusarium
13	G237	OE	Increased tolerance to Erysiphe
15	G409	OE	Increased tolerance to Erysiphe
17	G418	OE	Increased tolerance to Pseudomonas
19	G591	OE	Increased tolerance to Erysiphe
21	G525	OE	Increased tolerance to Pseudomonas
23	G545	OE	Increased susceptibility to Pseudomonas, Erysiphe and Fusarium
25	G865	OE	Increased susceptibility to Erysiphe and Botrytis
27 ·	G881	OE	Increased susceptibility to Erysiphe and Botrytis
29	G896	KO	Increased susceptibility to Fusarium
31	G378	OE	Increased resistance to Erysiphe
33	G569	OE	Decreased expression of defense genes
35	G558	OE	Increased expression of defense genes

For a particular overexpressor that shows an increased susceptibility to a pathogen, it may be more useful to select a plant with a decreased expression of the particular transcription factor. For a particular knockout that shows an increased susceptibility to a pathogen, it may be more useful to select a plant with an increased expression of the particular transcription factor.

Other than Fusarium oxysporum, Erysyphe orontii, the transgenic plants are more tolerant to Sclerotinia spp., soil-borne oomycetes, foliar oomycetes, Botrytis spp., Rhizoctonia spp, Verticillium dahliae/albo-atrum, Alternaria spp., rusts, Mycosphaerella spp, Fusarium solani, or the like. The transgenic plants are more resistant to fungal diseases such as rusts, smuts, wilts, yellows, root rot, leaf drop, ergot, leaf blight of potato, brown spot of rice, leaf blight, late blight, powdery mildew, downy mildew, and the like; viral diseases such as sugarcane mosaic, cassava mosaic, sugar beet yellows, plum pox, barley yellow dwarf, tomato yellow leaf curl, tomato spotted wilt virus, and the like; bacterial diseases such as citrus canker, bacterial leaf blight, bacterial will, soft rot of vegetables, and the like; nematode diseases such as root knot, sugar beet cyst nematode or the like.

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EXAMPLE IX. IDENTIFICATION OF HOMOLOGOUS SEQUENCES

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Homologous sequences from *Arabidopsis* and plant species other than *Arabidopsis* were identified using database sequence search tools, such as the Basic Local Alignment Search Tool (BLAST) (Altschul et al. (1990) <u>J. Mol. Biol.</u> 215:403-410; and Altschul et al. (1997) <u>Nucl. Acid Res.</u> 25: 3389-3402). The tblastx sequence analysis programs were employed using the BLOSUM-62 scoring matrix (Henikoff, S. and Henikoff, J. G. (1992) <u>Proc. Natl. Acad. Sci. USA</u> 89: 10915-10919).

Identified Arabidopsis homologous sequences are provided in Figure 2 and included in the Sequence Listing. The percent sequence identity among these sequences is as low as 47% sequence identity. Additionally, the entire NCBI GenBank database was filtered for sequences from all plants except Arabidopsis thaliana by selecting all entries in the NCBI GenBank database associated with NCBI taxonomic ID 33090 (Viridiplantae; all plants) and excluding entries associated with taxonomic ID 3701 (Arabidopsis thaliana). These sequences were compared to sequences representing genes of SEQ IDs Nos. 1-58 on 9/26/2000 using the Washington University TBLASTX algorithm (version 2.0a19MP). For each gene of SEQ IDs Nos. 1-58, individual comparisons were ordered by probability score (P-value), where the score reflects the probability that a particular alignment occurred by chance. For example, a score of 3.6e-40 is 3.6 x 10⁻⁴⁰. For up to ten species, the gene with the lowest P-value (and therefore the most likely homolog) is listed in Figure 3.

In addition to P-values, comparisons were also scored by percentage identity. Percentage identity reflects the degree to which two segments of DNA or protein are identical over a particular length. The ranges of percent identity between the non-Arabidopsis genes shown in Figure 3 and the Arabidopsis genes in the sequence listing are: SEQ ID No. 1: 38%-76%; SEQ ID No. 3: 36%-72%; SEQ ID No. 5: 51%-75%; SEQ ID No. 7: 37%-76%; SEQ ID No. 9: 48%-75%; SEQ ID No. 11: 31%-68%; SEQ ID No. 13: 59%-81%; SEQ ID No. 15: 49%-81%; SEQ ID No. 17: 53%-87%; SEQ ID No. 19: 48%-84%; SEQ ID No. 21: 73%-89%; SEQ ID No. 23: 52%-64%; SEQ ID No. 25: 48%-83%; SEQ ID No. 27: 35%-92%; SEQ ID No. 29: 56%-89%; SEQ ID No. 31: 50%-90%; SEQ ID No. 33: 50%-93%; SEQ ID No. 35: 52%-81%; SEQ ID No. 37: 75%-81%; SEQ ID No. 39: 35%-72%; SEQ ID No. 41: 55%-89%; SEQ ID No. 43: 56%-77%; SEQ ID No. 45: 34%-72%; SEQ ID No. 47: 51%-86%; SEQ ID No. 49: 46%-86%; SEQ ID No. 51: 58%-80%; SEQ ID No. 53: 46%-55%; SEQ ID No. 55: 84%-89%; and SEQ ID No. 57: 43%-71%.

The polynucleotides and polypeptides in the Sequence Listing and the identified homologous sequences may be stored in a computer system and have associated or linked with the sequences a function, such as that the polynucleotides and polypeptides are useful for modifying the pathogen tolerance of a plant.

All references, publications, patents and other documents herein are incorporated by reference in their entirety for all purposes. Although the invention has been described with reference to the embodiments and examples above, it should be understood that various modifications can be made without departing from the spirit of the invention.

What is claimed is:

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1. A transgenic plant with modified pathogen tolerance, which plant comprises a recombinant polynucleotide comprising a nucleotide sequence selected from the group consisting of:

- (a) a nucleotide sequence encoding a polypeptide comprising a sequence selected from SEQ ID Nos. 2N, where N=1-29, or a complementary nucleotide sequence thereof:
 - (b) a nucleotide sequence encoding a polypeptide comprising a conservatively substituted variant of a polypeptide of (a);
- (c) a nucleotide sequence comprising a sequence selected from those of SEQ ID Nos.2N-1, where N=1-29, or a complementary nucleotide sequence thereof;
 - (d) a nucleotide sequence comprising silent substitutions in a nucleotide sequence of (c);
 - (e) a nucleotide sequence which hybridizes under stringent conditions to a nucleotide sequence of one or more of: (a), (b), (c), or (d);
 - (f) a nucleotide sequence comprising at least 15 consecutive nucleotides of a sequence of any of (a)-(e);
 - (g) a nucleotide sequence comprising a subsequence or fragment of any of (a)-(f), which subsequence or fragment encodes a polypeptide that modifies a plant's pathogen tolerance;
 - (h) a nucleotide sequence having at least 31% sequence identity to a nucleotide sequence of any of (a)-(g);
 - (i) a nucleotide sequence having at least 60% identity sequence identity to a nucleotide sequence of any of (a)-(g);
- 25 (j) a nucleotide sequence which encodes a polypeptide having at least 31% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-29;
 - (k) a nucleotide sequence which encodes a polypeptide having at least 60% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-29; and
- (1) a nucleotide sequence which encodes a polypeptide having at least 65% sequence identity to a conserved domain of a polypeptide of SEQ ID Nos. 2N, where N=1-29.
 - 2. The transgenic plant of claim 1, further comprising a constitutive, inducible, or tissue-active promoter operably linked to said nucleotide sequence.
- 35 3. The transgenic plant of claim 1, wherein the plant is selected from the group consisting of: soybean, wheat, corn, potato, cotton, rice, oilseed rape, sunflower, alfalfa, sugarcane, turf, banana, blackberry, blueberry, strawberry, raspberry, cantaloupe, carrot,

cauliflower, coffee, cucumber, eggplant, grapes, honeydew, lettuce, mango, melon, onion, papaya, peas, peppers, pineapple, spinach, squash, sweet corn, tobacco, tomato, watermelon, rosaceous fruits, and vegetable brassicas.

- 5 4. An isolated or recombinant polynucleotide comprising a nucleotide sequence selected from the group consisting of:
 - (a) a nucleotide sequence encoding a polypeptide comprising a sequence selected from SEQ ID Nos. 2N, where N=1-29, or a complementary nucleotide sequence thereof;
- 10 (b) a nucleotide sequence encoding a polypeptide comprising a conservatively substituted variant of a polypeptide of (a);

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- (c) a nucleotide sequence comprising a sequence selected from those of SEQ ID Nos. 2N-1, where N=1-29, or a complementary nucleotide sequence thereof;
- (d) a nucleotide sequence comprising silent substitutions in a nucleotide sequence of (c);
- (e) a nucleotide sequence which hybridizes under stringent conditions to a nucleotide sequence of one or more of: (a), (b), (c), or (d);
- (f) a nucleotide sequence comprising at least 15 consecutive nucleotides of a sequence of any of (a)-(e);
- 20 (g) a nucleotide sequence comprising a subsequence or fragment of any of (a)-(f), which subsequence or fragment encodes a polypeptide having a biological activity that modifies a plant's pathogen tolerance;
 - (h) a nucleotide sequence having at least 31% sequence identity to a nucleotide sequence of any of (a)-(g);
- 25 (i) a nucleotide sequence having at least 60% identity sequence identity to a nucleotide sequence of any of (a)-(g);
 - (j) a nucleotide sequence which encodes a polypeptide having at least 31% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-29;
 - (k) a nucleotide sequence which encodes a polypeptide having at least 60% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-29; and
 - (1) a nucleotide sequence which encodes a conserved domain of a polypeptide having at least 65% sequence identity to a conserved domain of a polypeptide of SEQ ID Nos. 2N, where N=1-29.
- The isolated or recombinant polynucleotide of claim 4, further comprising a constitutive, inducible, or tissue-active promoter operably linked to the nucleotide sequence.

6. A cloning or expression vector comprising the isolated or recombinant polynucleotide of claim 4.

- 7. A cell comprising the cloning or expression vector of claim 6.
- A transgenic plant comprising the isolated or recombinant polynucleotide of claim 4.
- 9. A composition produced by one or more of:
 - (a) incubating one or more polynucleotide of claim 4 with a nuclease;
- 10 (b) incubating one or more polynucleotide of claim 4 with a restriction enzyme;
 - (c) incubating one or more polynucleotide of claim 4 with a polymerase;
 - (d) incubating one or more polynucleotide of claim 4 with a polymerase and a primer;
 - (e) incubating one or more polynucleotide of claim 4 with a cloning vector, or
 - (f) incubating one or more polynucleotide of claim 4 with a cell.

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- 10. A composition comprising two or more different polynucleotides of claim 4.
- 11. An isolated or recombinant polypeptide comprising a subsequence of at least about 15 contiguous amino acids encoded by the recombinant or isolated polynucleotide of claim 4.

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- 12. A plant comprising an isolated polypeptide of claim 11.
- 13. A method for producing a plant having a modified pathogen tolerance, the method comprising altering the expression of the isolated or recombinant polynucleotide of claim 4 or the expression levels or activity of a polypeptide of claim 11 in a plant, thereby producing a modified plant, and selecting the modified plant for improved pathogen tolerance thereby providing the modified plant with a modified pathogen tolerance.
 - 14. The method of claim 13, wherein the polynucleotide is a polynucleotide of claim 4.

- 15. A method of identifying a factor that is modulated by or interacts with a polypeptide encoded by a polynucleotide of claim 4, the method comprising:
 - (a) expressing a polypeptide encoded by the polynucleotide in a plant; and
- (b) identifying at least one factor that is modulated by or interacts with the polypeptide.

16. The method of claim 15, wherein the identifying is performed by detecting binding by the polypeptide to a promoter sequence, or detecting interactions between an additional protein and the polypeptide in a yeast two hybrid system.

- 5 17. The method of claim 15, wherein the identifying is performed by detecting expression of a factor by hybridization to a microarray, subtractive hybridization or differential display.
 - 18. A method of identifying a molecule that modulates activity or expression of a polynucleotide or polypeptide of interest, the method comprising:
- (a) placing the molecule in contact with a plant comprising the polynucleotide or polypeptide encoded by the polynucleotide of claim 4; and,
 - (b) monitoring one or more of:
 - (i) expression level of the polynucleotide in the plant;
 - (ii) expression level of the polypeptide in the plant;
 - (iii) modulation of an activity of the polypeptide in the plant; or
 - (iv) modulation of an activity of the polynucleotide in the plant.
 - 19. An integrated system, computer or computer readable medium comprising one or more character strings corresponding to a polynucleotide of claim 4, or to a polypeptide encoded by the polynucleotide.
 - 20. The integrated system, computer or computer readable medium of claim 19, further comprising a link between said one or more sequence strings to a modified plant pathogen tolerance phenotype.

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- 21. A method of identifying a sequence similar or homologous to one or more polynucleotides of claim 4, or one or more polypeptides encoded by the polynucleotides, the method comprising:
 - (a) providing a sequence database; and,
- (b) querying the sequence database with one or more target sequences corresponding to the one or more polynucleotides or to the one or more polypeptides to identify one or more sequence members of the database that display sequence similarity or homology to one or more of the one or more target sequences.
- 35 22. The method of claim 21, wherein the querying comprises aligning one or more of the target sequences with one or more of the one or more sequence members in the sequence database.

23. The method of claim 21, wherein the querying comprises identifying one or more of the one or more sequence members of the database that meet a user-selected identity criteria with one or more of the target sequences.

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- 24. The method of claim 21, further comprising linking the one or more of the polynucleotides of claim 4, or encoded polypeptides, to a modified plant pathogen tolerance phenotype.
- 10 25. A plant comprising altered expression levels of an isolated or recombinant polynucleotide of claim 4.
 - 26. A plant comprising altered expression levels or the activity of an isolated or recombinant polypeptide of claim 11.

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27. A plant lacking a nucleotide sequence encoding a polypeptide of claim 11.

Figure 1

SEQ ID No.	GID	cDNA or protein	conserved domain
1	G188	cDNA	
2	G188	protein	175-222
3	G616	cDNA	
4	G616	protein	39-95
5	G19	cDNA	
6	G19	protein ·	76-145
7	G261	cDNA	
8	G261	protein-	16-104
9	G28	cDNA	
10	G28	protein	145-213
11	G869	cDNA	
12	G869	protein	109-177
13	G237	cDNA	
14	G237	protein	11-113
15	G409	cDNA	
16	G409	protein	64-124
17	G418	cDNA	
18	G418	protein	500-560
19	G591	cDNA	
20	G591	protein	143-240
21	G525	cDNA	
22	G525	protein	23-167
23	G545	cDNA	
24	G545	protein	82-102, 136-154
25	G865	cDNA	
26	G865	protein	36-103
27	G881	cDNA	
28	G881	protein	176-233
29	G896	cDNA	
30	G896	protein	18-39
31	G378	cDNA	
32	G378	protein	196-237
33	G569	cDNA	
34	G569	protein	90-153
35	G558	cDNA	
36	G558	protein	45-105

Figure 2

SEQ ID No.	GID	homolog	cDNA or protein	conserved domain
37	G1396	homolog of G1394	cDNA	
38	G1396	homolog of G1394	protein	entire protein
39	G265	homolog of G261	cDNA	
40	G265	homolog of G261	protein	14-105
41	G1006	homolog of G28	cDNA	
42	G1006	homolog of G28	protein	114-182
43	G1309	homolog of G237	cDNA	
44	G1309	homolog of G237	protein	9-114
45	G2550	homolog of G418	cDNA	
46	G2550	homolog of G418	protein	348-408
47	G965	homolog of G418	cDNA	
48	G965	homolog of G418	protein	423-486
49	G793	homolog of G591	cDNA	
50	G793	homolog of G591	protein	151-206
51	G764	homolog of G525	cDNA	
52	G764	homolog of G525	protein	27-171 -
53	G350	homolog of G545	cDNA	
54	G350	homolog of G545	protein	91-113,150-170
55	G986	homolog of G881	cDNA	
56	G986	homolog of G881	protein	146-203
57	G1349	homolog of G896	cDNA	
58	G1349	homolog of G896	protein	13-63

Figure 3A

SEQ ID No.	GID	Genbank NID	P-value	Species
1	G188	7779802	5.20E-36	Lotus japonicus
1	G188	7284340	2.10E-34	Glycine max
1	G188	9361307	1.20E-27	Triticum aestivum
1	G188	7340336	1.10E-22	Oryza sativa
1	G188	6529152	3.60E-22	Lycopersicon esculentum
- 1	G188	8748477	7.70E-21	Medicago truncatula
1	G188	5456433	7.10E-14	Zea mays
1	G188	9302479	1.60E-12	Sorghum bicolor
1	· G188	6696287	4.10E-12	Pinus taeda
1	G188	562242	9.00E-12	Brassica rapa
3	G616	7719440	8.30E-37	Lotus japonicus
3	G616	7692230	5.90E-33	Glycine max
3	G616	7501307	1.10E-21	Gossypium arboreum
3	G616	8071090	1.50E-21	Solanum tuberosum
3	G616	8858771	1.50E-21	Oryza sativa
3	G616	5047315	1.50E-21	Gossypium hirsutum
3	G616	6358532	5.80E-20	
3	G616	2826867		Antirrhinum graniticum
3	G616		7.00E-20	Antirrhinum majus
3		6358535	7.40E-20	Antirrhinum majus subsp. linkianum
5	G616	6358538	7.50E-20	Antirrhinum braun-blanquetii
	G19	8789223	2.80E-34	Citrus x paradisi
-5	G19	9434234	4.50E-34	Lycopersicon esculentum
5	G19	7478682	1.30E-30	Glycine max
5	G19	6654934	1.20E-28	Medicago truncatula
5	G19	3264766	5.50E-26	Prunus armeniaca
5	G19	7624302	8.30E-26	Gossypium arboreum
5	G19	9425363	2.90E-25	Triticum aestivum
5	G19	688579	3.60E-25	Ricinus communis
. 5	G19	9419304	6.00E-25	Hordeum vulgare
5	G19	7720316	8.80E-25	Lotus japonicus
7	G261	5821137	5.10E-93	Nicotiana tabacum
7	G261	7158881	8.80E-86	Medicago sativa
7	G261	886741	1.00E-73	Zea mays
7	G261	5900449	5.20E-47	Lycopersicon esculentum
7	G261	7561318	1.20E-46	Medicago truncatula
7	G261	19491	1.70E-42	Lycopersicon peruvianum
7	G261	7233914	3.50E-41	Glycine max
7	G261	4528238	9.00E-41	Citrus unshiu
7	G261	8903922	4.00E-39	Hordeum vulgare
7	G261	9251913	1.90E-36	Solanum tuberosum
9	G28	7528275	4.20E-62	Mesembryanthemum crystallinum
9	G28	6654776	1.20E-57	Medicago truncatula
9	G28	790362	2.30E-54	Nicotiana tabacum
9	G28	8809570	8.00E-54	Nicotiana sylvestris
9	G28	3342210	8.40E-54	Lycopersicon esculentum
9	G28	6566281	8.40E-47	Glycine max
9	G28	7627061	8.40E-47	Gossypium arboreum
9	G28	7324479	2.00E-44	Lycopersicon pennellii
9	G28	6478844	1.80E-35	Matricaria chamomilla
9	G28	7273972	7.80E-29	Oryza sativa
11	G869	2213784	1.30E-19	Lycopersicon esculentum

Figure 3B

SEQ ID No.	GID	Genbank NID	P-value	Species
				Oryza sativa
11	G869	8570080	4.20E-18	
11	G869	7560260	1.50E-17	Medicago truncatula
11	G869	7534890	5.20E-14	Sorghum bicolor
11	G869	6455322	1.10E-13	Glycine max
11	G869	9362061	2.70E-13	Triticum aestivum
11	G869	7788764	5.70E-13	Lotus japonicus
11	G869	7624302	2.50E-12	Gossypium arboreum
11	G869	3858036	2.80E-12	Populus balsamifera subsp. trichocarpa
13	G237	8283916	4.70E-42	Glycine max
13	G237	9361969	8.30E-41	Triticum aestivum
13	G237	4753385	4.10E-39	Zea mays
13	G237	7535969	4.10E-33	Sorghum bicolor
13	G237	7566043	9.30E-33	Medicago truncatula
13	G237	7339127	2.00E-32	Lycopersicon esculentum
13	G237	5860031	1.10E-28	Pinus taeda
13	G237	7776223	2.20E-28	Lotus japonicus
13	G237	6850206	5.10E-28	Oryza sativa
13	G237	5048991	8.50E-28	Gossypium hirsutum
15	G409	6654773	6.10E-57	Medicago truncatula
15	G409	6531235	2.00E-56	Lycopersicon esculentum
15	G409	7924152	1.10E-47	Glycine max
15	G409	. 5006854	6.50E-43.	Oryza sativa
15	G409	8098529	2.10E-41	Hordeum vulgare
15	G409	767697	1.40E-37	Daucus carota
15	G409	8328991	3.30E-37	Mesembryanthemum crystallinum
15	G409	7415613	1.40E-32	Physcomitrella patens
15	G409	7785121	2.80E-32	Lotus japonicus
15	G409	6916941	4.80E-32	Lycopersicon pennellii
17	G418	7239156 ⁻	1.90E-123	Malus x domestica
17	G418	5892190	2.00E-62	Lycopersicon esculentum
17	G418	7628137	8.70E-58	Gossypium arboreum
17	G418	9205496	3.90E-51	Glycine max
17	G418	6069643	1.50E-45	Oryza sativa
17	G418	7562931	6.90E-45	Medicago truncatula
17	G418	7781695	5.50E-40	Lotus japonicus
17	G418	9298824	7.80E-34	Sorghum bicolor
17	G418	9428023	3.90E-32	Triticum aestivum
17	G418	7244366	1.30E-31	Mentha x piperita
19	G591	7646333	1.90E-55	Lycopersicon esculentum
19	G591	7924288	4.10E-53	Glycine max
19	G591	7722838	1.10E-41	Lotus japonicus
19	G591	5804781	1.40E-24	Nicotiana tabacum
19	G591	9198126	2.50E-23	Medicago truncatula
19	G591	427677	9.50E-15	Oryza sativa
19	G591	7624745	1.80E-14	Gossypium arboreum
19	G591	7535578	8.70E-14	Sorghum bicolor
19	G591	5915205	1.30E-11	Zea mays
19	G591	9249806	2.60E-11	Solanum tuberosum
21	G525	4384535	5.60E-61	Lycopersicon esculentum
21	G525	6454868	2.00E-58	Glycine max
21	G525	6066594	9.30E-54	Petunia x hybrida
21	G525	4977542	8.60E-51	Oryza sativa

Figure 3C

SEQ ID No.	GID	Genbank NID	P-value	Species
21	G525	9361647	2.50E-50	Triticum aestivum
21	G525	4218536	5.20E-50	Triticum sp.
21	G525	6732159	5.20E-50	Triticum monococcum
21	G525	5343151	2.70E-49	Zea mays
21	G525	5049217	4.20E-48	Gossypium hirsutum
21	G525	8708684	8.90E-48	Hordeum vulgare
· 23	G545	4666359	8.30E-55	Datisca glomerata
23	G545	7228328	3.70E-52	Medicago sativa
23	. G545	1763062	1.30E-51	Glycine max
23	G545	7206360	3.10E-44	Medicago truncatula
23	G545	7626808	9.60E-40	Gossypium arboreum
23	G545	439492	3.90E-39	Petunia x hybrida
23	G545	4382658	1.70E-38	Lycopersicon esculentum
23	G545	8486215	8.70E-38	Euphorbia esula
23	G545	7322653	6.80E-37	Lycopersicon hirsutum
23	G545	7785845	1.10E-33	Lotus japonicus
25	G865	9417297	1.70E-32	Triticum aestivum
25	G865	7206394	4.90E-29	Medicago truncatula
25	G865	7796858	5.70E-27	Glycine max
25	G865	4387560	9.20E-25	Lycopersicon esculentum
25	G865	569065	1.50E-23	Oryza sativa
25	G865	7788764	4.10E-23	Lotus japonicus
25	G865	790362	8.40E-22	Nicotiana tabacum
25	G865	7528275	5.90E-21	Mesembryanthemum crystallinum
25	G865	3264766	8.80E-20	Prunus armeniaca
25	G865	8098026	2.00E-19	Hordeum vulgare
27	G881	5820418	9.80E-29	Glycine max
27	G881	8440065	1.00E-27	Gossypium hirsutum
27	G881	4380578	1.50E-27	Lycopersicon esculentum
27	G881	9199620	2.70E-27	Medicago truncatula
27	G881	6472584	2.20E-24	Nicotiana tabacum
27	G881	9250698	3.20E-24	Solanum tuberosum
27	G881	8205146	5.20E-21	Oryza sativa
27	G881	1159878	8.20E-17	Avena fatua
27	G881	9299778	2.70E-16	Sorghum bicolor
27	G881	9444636	1.10E-14	Triticum aestivum
29	G896	9410462	1.90E-101	Hordeum vulgare
29	G896	7628908	3.60E-82	Gossypium arboreum
29	G896	7244408	1.80E-79	Mentha x piperita
29	G896	5046180	2.10E-73	Gossypium hirsutum
29	G896	7678652	1.10E-63	Lotus japonicus
29	G896	8286031	1.40E-60	Glycine max
29	G896	5888938	4.50E-58	Lycopersicon esculentum
29	G896	9298238	9.20E-54	Sorghum bicolor
29	G896	7566414	8.00E-52	Medicago truncatula
29	G896	8845076	1.00E-46	Triticum aestivum
31	G378	5270028		
31	G378		5.10E-73 4.10E-58	Lycopersicon esculentum
31		5048335		Gossypium hirsutum Oryza sativa
31	G378 G378	7239521	5.90E-42	Glycine max
		5606120 3853800	6.80E-36	Populus tremula x Populus tremuloides
31	G378	3853800	3.20E-30	· · · · · · · · · · · · · · · · · · ·
31	G378	7659983	1.70E-23	Sorghum bicolor

Figure 3D

SEQ ID No.	GID	Genbank NID	P-value	Species
31	G378	6626305	1.10E-21	Zea mays
31	G378	9412941	9.40E-19	Triticum aestivum
31	G378	3242033	4.30E-17	Mesembryanthemum crystallinum
31	G378	7626259	7.70E-13	Gossypium arboreum
33	G229	7337390	6.60E-51	Lycopersicon esculentum
33	G229	9823237	3.60E-50	Hordeum vulgare
33	G229	7244424	4.90E-50	Mentha x piperita
33	G229	7776053	1.70E-49	Lotus japonicus
33	G229	2921335	5.80E-48	Gossypium hirsutum
33	G229	1491932	4.50E-47	Zea mays
33	G229	6455590	2.80E-44	Glycine max
33	G229	6020191	2.00E-41	Pinus taeda ·
33	G229	10697236	4.20E-41	Oryza sativa
33	G229	7765706	5.10E-41	Medicago truncatula
35	G663	7673087	5.10E-43	Petunia integrifolia
35	G663	9508051	3.00E-41	Lycopersicon esculentum
35	G663	7673091	3.30E-41	Petunia x hybrida
35	G663	7673097	2.40E-36	Petunia axillaris
35	G663	5048991	1.20E-33	Gossypium hirsutum
35	G663	6455590	2.50E-31	Glycine max
35	G663	7560175	1.90E-27	Medicago truncatula
35	G663	7244424	4.10E-26	Mentha x piperita
35	G663	9954117	3.40E-25	Solanum tuberosum
35	G663	6020191	3.60E-25	Pinus taeda
37	G1396	498704	5.20E-22	Spinacia oleracea
37	G1396	7502400	1.20E-21	Gossypium arboreum
37	G1396	3857536	3.40E-21	Populus balsamifera subsp. trichocarpa
37	G1396	4385300	1.20E-20	Lycopersicon esculentum
37	G1396	6917249	1.50E-20	Lycopersicon pennellii
37	G1396	6915979	1.70E-20	Glycine max
37	G1396	7674530	2.70E-20	Medicago truncatula
37	G1396	8090319	3.40E-20	Sorghum bicolor
37	G1396	3592182	9.10E-20	Oryza sativa
37	G1396	6654124	1.10E-19	Zea mays
39	G265	5821137	6.50E-83	Nicotiana tabacum
39	G265	7158881	3.80E-79	Medicago sativa
39	G265	886741	1.60E-70	Zea mays
39	G265	5900449	5.60E-43	Lycopersicon esculentum
39	G265	8903922	8.20E-43	Hordeum vulgare
39	G265	7561318	2.10E-41	Medicago truncatula
39	G265	9204445	5.30E-36	Glycine max
. 39	G265	4528238	5.40E-36	Citrus unshiu
39	G265	19489	2.10E-35	Lycopersicon peruvianum
39	G265	9251913	2.00E-32	Solanum tuberosum
41	G1006	7528275	2.70E-51	Mesembryanthemum crystallinum
41	G1006	3342210	4.90E-49	Lycopersicon esculentum
41	G1006	6654776	1.90E-48	Medicago truncatula
41	G1006	790362	2.30E-47	Nicotiana tabacum
41	G1006	8809570	2.00E-46	Nicotiana sylvestris
41	G1006	7627061	6.40E-41	Gossypium arboreum
	G1006	7324479	1.20E-35	Lycopersicon pennellii
41		6478844	1.80E-35	Matricaria chamomilla
41	G1006	04/0044	1.00=*33	ITICA ICATIO OTICITIONINA

Figure 3E

SEQ ID No.	GID	Genbank NID	P-value	Species
41	G1006	6566281	1.30E-34	Glycine max
41	G1006	4716624	3.80E-28	Oryza sativa
43	G1309	9361969	2.40E-45	Triticum aestivum
43	G1309	7566043	9.60E-35	Medicago truncatula
43	G1309	5891104	2.20E-31	Lycopersicon esculentum
43	G1309	5860031	2.10E-30	Pinus taeda
43	G1309	5049507	6.20E-30	Gossypium hirsutum
43	G1309	5139805	1.30E-29	Glycine max
43	G1309	6850206	2.50E-29	Oryza sativa
43	G1309	7721017	3.40E-29	Lotus japonicus
43	G1309	8368245	5.20E-28	Zea mays
43	G1309	20560	9.50E-27	Petunia x hybrida
45	G2550	4380729	2.80E-51	Lycopersicon esculentum
45	G2550	5667196	2.20E-49	Oryza sativa
45	G2550	8669454	1.40E-48	Glycine max
45	G2550	9298824	1.50E-48	Sorghum bicolor
45	G2550	7239156	9.90E-46	Malus x domestica
45	G2550	7570704	5.70E-45	Medicago truncatula
45	G2550	7628137	3.30E-42	Gossypium arboreum
. 45	G2550	7244366		Mentha x piperita
45			6.00E-41	Triticum aestivum
45	G2550	9428023	4.70E-40	Solanum tuberosum
45	G2550	9250642	3.50E-39	
	G965	7239156	3.10E-126	Malus x domestica
47	G965	5892190	2.00E-62	Lycopersicon esculentum
47	G965	7628137	1.60E-56	Gossypium arboreum
47	G965	9205496	2.60E-49	Glycine max
47	G965	6069643	1.70E-45	Oryza sativa
47	G965	7562931	2.50E-44 1.60E-41	Medicago truncatula Lotus japonicus
47	G965 G965	7781695 9298824	6.30E-33	Sorghum bicolor
47	G965	9428023	1.50E-31	Triticum aestivum
47				Mentha x piperita
	G965	7244366	1.20E-29	
49	G793	6976712	3.60E-43	Lycopersicon esculentum
49	G793	7924288	2.00E-41	Glycine max
49	G793	7614163	3.90E-34	Lotus japonicus
49	G793	9198126	5.70E-23	Medicago truncatula
49	G793	5804781	1.10E-22	Nicotiana tabacum
49	G793	7535578	1.60E-14	Sorghum bicolor
49	G793	427677	6.10E-14	Oryza sativa
49	G793	5915205	2.90E-10	Zea mays
49	G793	9249806	4.20E-10	Solanum tuberosum
49	G793 .	7624745	1.30E-09	Gossypium arboreum
51	G764	4384535	7.00E-70	Lycopersicon esculentum
51	G764	5049217	1.80E-65	Gossypium hirsutum
51	G764	6454868	1.90E-64	Glycine max
51	G764	6066594	5.20E-59	Petunia x hybrida
51	G764	4218536	2.30E-52	Triticum sp.
51	G764	6732159	2.30E-52	Triticum monococcum
51	G764	9361647	7.50E-52	Triticum aestivum
51	G764	4977542	4.10E-49	Oryza sativa
51	G764	6799764	4.40E-49	Medicago truncatula
51	G764	9296257	1.00E-48	Sorghum bicolor

Figure 3F

SEQ ID No.	GID	Genbank NID	P-value	Species
53	G350	439492	5.20E-53	Petunia x hybrida
53	G350	7228328	8.90E-51	Medicago sativa
53	G350	4666359	3.10E-48	Datisca glomerata
53	G350	1763062	8.30E-48	Glycine max
53	G350	7626808	9.10E-44	Gossypium arboreum
53	G350	7206360	2.20E-43	Medicago truncatula
53	G350	2981168	2.10E-38	Nicotiana tabacum
53	G350	7322653	2.00E-37	Lycopersicon hirsutum
53	G350	5276755	2.40E-37	Lycopersicon esculentum
53	G350	2058503	1.10E-31	Brassica rapa
55	G986	6472584	1.00E-34	Nicotiana tabacum
55	G986	8440065	8.80E-33	Gossypium hirsutum
55	G986	4385167	1.50E-32	Lycopersicon esculentum
55	G986	8205146	5.50E-30	Oryza sativa
55	G986	5820418	8.80E-26	Glycine max
55	G986	1159878	2.30E-23	Avena fatua
55	G986	9250698	4.60E-22	Solanum tuberosum
55	G986	9413507	7.90E-21	Triticum aestivum
55	G986	7748539	2.30E-20	Lotus japonicus
55	G986	9199620	1.30E-16	Medicago truncatula
57	G1349	8904043	1.50E-47	Hordeum vulgare
57	G1349	7244408	2.40E-47	Mentha x piperita
57	G1349	8286031	3.60E-46	Glycine max
57	G1349	9298238	9.10E-36	Sorghum bicolor
. 57	G1349	7628908	4.70E-34	Gossypium arboreum
57	G1349	5046180	1.50E-33	Gossypium hirsutum
57	G1349	5888938	1.30E-30	Lycopersicon esculentum
57	G1349	5043924	6.20E-30	Pinus taeda
57	G1349	8845076	4.40E-29	Triticum aestivum
57	G1349	7678652	4.20E-27	Lotus japonicus

MBI15 Sequence Listing.ST25 SEQUENCE LISTING

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Glu Asn Leu Ser Ser Asp Leu Trp Ala Trp Arg Lys Tyr Gly Gln Lys 165 170 175

Pro Ile Lys Gly Ser Pro Tyr Pro Arg Asn Tyr Tyr Arg Cys Ser Ser 180 185 190

Ser Lys Gly Cys Leu Ala Arg Lys Gln Val Glu Arg Ser Asn Leu Asp 195 200 205

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aac aac cgg tta agc cgg tgg cat cac aat tct tcc cgg at at acc and sent Arg Leu Ser Arg Trp His His Asn Ser Ser Arg Ile Ile Arg 25 30 gtt tca cga gct tcc ggt ggt aaa gat cga cac agc aaa gtc ttg act Val Ser Arg Ala Ser Gly Gly Lys Asp Arg His Ser Lys Val Leu Thr 35 40 45 tct aaa gga cca cgt gac cgt ggt cgg tta tca gtc tcc acc gct Ser Lys Gly Pro Arg Asp Arg Arg Val Arg Leu Ser Val Ser Thr Ala 60 55 ctt caa ttc tat gat ctt caa gat cgg tta ggt tat gat caa cct agc Leu Gln Phe Tyr Asp Leu Gln Asp Arg Leu Gly Tyr Asp Gln Pro Ser 75 aaa gct gtt gaa tgg tta atc aaa gct ggt gaa gat tca atc tct, gag 10 19 19 10 10 10 10 10 10 10 10 10 10 10 10 10	gagatcta atg aag aat aac aac aat ggc gac gtt gtg gat aac gaa gtg Met Lys Asn Asn Asn Gly Asp Val Val Asp Asn Glu Val	170
gtt tca cga gct tcc ggt ggt aaa gat cga cac agc aaa gtc ttg act Val Ser Arg Ala Ser Gly Gly Lys Asp Arg His Ser Lys Val Leu Thr 45 tct aaa gga cca cgt gac cgt cgt gtc cgg tta tca gtc tcc acc gct Ser Lys Gly Pro Arg Asp Arg Val Arg Leu Ser Val Ser Thr Ala 60 ctt caa ttc tat gat ctt caa gat cgg tta ggt tat gat caa cct agc Leu Gln Phe Tyr Asp Leu Gln Asp Arg Leu Gly Tyr Asp Gln Pro Ser 70 aaa gct gtt gaa tgg tta atc aaa gct gct gaa gat tca atc tct. gag Lys Ala Val Glu Trp Leu Ile Lys Ala Ala Glu Asp Ser Ile Ser Glu 90 ctt cct tca ctc aac aac act cat ttt ccg acc gat gac gag aat cac Leu Pro Ser Leu Asn Asn Thr His Phe Pro Thr Asp Asp Glu Asn His 110 cag aat cag aca tta aca aca gtt gct gat aat tcc ttg tct aaa tct Lys Ala Cys Ser Ser Asp Ser Asp Thr Ser Lys Asn Ser Ser Gly Leu Ser 1130 cta tca aga tcg gag ctt aga gat aaa gct aga gag agg ggt ttg ttg tct Lys Asn Ser Asp Ser Glu Arg Asp Lys Ala Arg Glu Arg Thr Ala Lys Glu Thr Lys Glu Arg Asp His Asn His Thr Ser Phe 165 acg gat ttg tta aat tcc ggt tca gat ccg gtt aac tca acc acc ttcg ttt aga aca gat tag aca gat aca cac acc acc ttg ttt Arg Ala Arg Glu Arg	Asn Asn Arg Lett Ser Arg 129 125 25 . 30	218
tct aaa gga cca cgt gac cgt gtc cgg tta tca gtc tca gtc table ser Lys Gly Pro Arg Asp Arg Arg Val Arg Leu Ser Val Ser Thr Ala 50 ctt caa ttc tat gat ctt caa gat cgg tta ggt tat gat caa cct agc Arg Leu Gln Phe Tyr Asp Leu Gln Asp Arg Leu Gly Tyr Asp Gln Pro Ser 75 aaa gct gtt gaa tgg tta atc aaa gct gct gaa gat tca atc tct gag Lys Ala Val Glu Trp Leu Ile Lys Ala Ala Glu Asp Ser Ile Ser Glu 85 ctt cct tca ctc aac aac act cat ttt ccg acc gat gac gag aat cac cac Leu Pro Ser Leu Asn Asn Thr His Phe Pro Thr Asp Asp Glu Asn His 100 cag aat cag aca tta aca aca gtt gct gct aat tcc ttg tct aaa tct Cal Lys Ser 115 gct tgt agt agc aat tca gac acg agc aag aac tct tct ggt Lys Ser 115 gct tgt agt agc aat tca gac acg acc acg agc aag aac tct tct ggt tgt can acc Cys Ser Ser Asn Ser Asp Thr Ser Lys Asn Ser Ser Glu Leu Ser Lys Asn Ser Ser Glu Leu Ser Arg Ser Glu Leu Arg Asp Lys Ala Arg Glu Arg A	gtt tca cga gct tcc ggt ggt aaa gat cga cac agc aaa gtc ttg act Val Ser Arg Ala Ser Gly Gly Lys Asp Arg His Ser Lys Val Leu Thr	266
Ctt caa ttc tat gat ctt caa gat cgg tta ggt tat ggt tat gat ctc car college of the college of th	Ser Lys Gly Pro Arg Asp Arg 115 55 60	314
Lys Ala Val Glu Trp Leu Ile Lys Ala Ala Glu Asp Ser Ile Ser Glu 80 ctt cct tca ctc aac aac act cat ttt ccg acc gat gac gag aat cac Leu Pro Ser Leu Asn Asn Thr His Phe Pro Thr Asp Asp Glu Asn His 105 cag aat cag aca tta aca aca gtt gct gct aat tcc ttg tct aaa tct Gln Asn Gln Thr Leu Thr Thr Val Ala Ala Asn Ser Leu Ser Lys Ser 125 gct tgt agt agc aat tca gac acg acg aag aac tct tct ggt tgt ser Ala Cys Ser Ser Asn Ser Asp Thr Ser Lys Asn Ser Ser Gly Leu Ser 130 tta tca aga tcg gag ctt aga gat aaa gct aga gag agg gct aga gag Lys Ala Arg Glu Arg Ala Arg	Leu Gln Phe Tyr Asp Leu Gli Asp 123 75	362
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cag aat cag aca tta aca aca gtt gct gct aat tcc ttg tct aaa tct Sof Gln Asn Gln Thr Leu Thr Thr Val Ala Ala Asn Ser Leu Ser Lys Ser 125 gct tgt agt agc aat tca gac acg agc aag aac tct tct ggt ttg tct Ala Cys Ser Ser Asn Ser Asp Thr Ser Lys Asn Ser Ser Gly Leu Ser 135 tta tca aga tcg gag ctt aga gat aaa gct aga gag agg gct aga gag Got Leu Ser Arg Ser Glu Leu Arg Asp Lys Ala Arg Glu Arg Ala Arg Glu 155 aga aca gct aaa gag acc aag gaa aga gat cat aac cac act tcg ttt Arg Thr Ala Lys Glu Thr Lys Glu Arg Asp His Asn His Thr Ser Phe 160 acg gat ttg tta aat tcc ggt tca gat ccg gtt aac tca aac cgg caa 180 acg gat ttg tta aat tcc ggt tca gat ccg gtt aac tca aac cgg caa 180 Thr Asp Leu Leu Asn Ser Gly Ser Asp Pro Val Asn Ser Asn Arg Gln 190 175	Leu Pro Ser Leu Ash Ash III Mas 105	458
gct tgt agt agc aat tca gac acg agc aag aac tct tct ggt tca gat ccg gt taac tca aga cac acg agc aag aac tct tct ggt tca gat ccg gat ttg tta aat tcc ggt tca gat ccg gt aac tca aac cac acc tcg ttt acg gat ttg tta aat tcc ggt tca gat ccg gt aac tca aac ccg caa Thr Asp Leu Asn Ser Gly Ser Asp Pro Val Asn Ser Asn Arg Gln 185 186 187 188 188 188 188 188 188	cag aat cag aca tta aca aca gtt gct gct aat tcc ttg tct aaa tct Gln Asn Gln Thr Leu Thr Thr Val Ala Ala Asn Ser Leu Ser Lys Ser	506
tta tca aga tcg gag ctt aga gat aaa gct aga gag gag gag gag get aga gag gag gag gag gag gag gag gag ga	Ala Cys Ser Ser Ash Set Asp 1125 140	554
acg gat ttg tta aat tcc ggt tca gat ccg gtt aac tca aac cgg caa acg gat ttg tta aat tcc ggt tca gat ccg gtt aac tca aac cgg caa Thr Asp Leu Leu Asn Ser Gly Ser Asp Pro Val Asn Ser Asn Arg Gln 185 180 175 180 746	Leu Ser Arg Ser Glu beu Arg 755 155	602
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Trp Met Ala Ser Ala Plo Ser Ser 200 205	tgg atg gct tca gct cct tct tca tct cca atg gag tat ttc agt tcg Trp Met Ala Ser Ala Pro Ser Ser Ser Pro Met Glu Tyr Phe Ser Ser 195 200 205	746

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aat tot cat cot tto toa toa ato too gat cat cat cat cat cot Asn Ser His Pro Phe Ser Ser Ile Ser Asp His His His His Pro 225 230 235	842
cat cat cag cat caa gag ttt tca ttc gtt ccc gac cat ttg ata tca His His Gln His Gln Glu Phe Ser Phe Val Pro Asp His Leu Ile Ser 240 245 250	890
ccg gca gaa tcc aac ggc gga gca ttc aat ctt gat ttt aat atg tca Pro Ala Glu Ser Asn Gly Gly Ala Phe Asn Leu Asp Phe Asn Met Ser 255 260 265 270	938
aca ccc tcc ggc gcc gga gct gcc gtc tcc gcc gca tca ggt ggc ggc Thr Pro Ser Gly Ala Gly Ala Ala Val Ser Ala Ala Ser Gly Gly Gly 275 280 285	986
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cat cag tca ttc ctc gct aat cta cag agg ttt cca aca tca gaa agt His Gln Ser Phe Leu Ala Asn Leu Gln Arg Phe Pro Thr Ser Glu Ser 305 310 315	1082
gga gga ggt cca cag ttc tta ttc ggt gca ctg cct gca gag aat cac Gly Gly Pro Gln Phe Leu Phe Gly Ala Leu Pro Ala Glu Asn His 320 325 330	1130
cac cac aat cac cag ttt cag ctt tac tat gaa aat gga tgc aga aac His His Asn His Gln Phe Gln Leu Tyr Tyr Glu Asn Gly Cys Arg Asn 335 340 345 350	1178
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Gly Pro Arg Asp Arg Arg Val Arg Leu Ser Val Ser Thr Ala Leu Gln 50 55

Phe Tyr Asp Leu Gln Asp Arg Leu Gly Tyr Asp Gln Pro Ser Lys Ala 65 70 75 80

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- Val Glu Trp Leu Ile Lys Ala Ala Glu Asp Ser Ile Ser Glu Leu Pro 85 90 95
- Ser Leu Asn Asn Thr His Phe Pro Thr Asp Asp Glu Asn His Gln Asn 100 105 110
- Gln Thr Leu Thr Thr Val Ala Ala Asn Ser Leu Ser Lys Ser Ala Cys 115 120 125
- Ser Ser Asn Ser Asp Thr Ser Lys Asn Ser Ser Gly Leu Ser Leu Ser 130 135 140
- Arg Ser Glu Leu Arg Asp Lys Ala Arg Glu Arg Ala Arg Glu Arg Thr 145 150 155 160
- Ala Lys Glu Thr Lys Glu Arg Asp His Asn His Thr Ser Phe Thr Asp 165 170 175
- Leu Leu Asn Ser Gly Ser Asp Pro Val Asn Ser Asn Arg Gln Trp Met 180 185
- Ala Ser Ala Pro Ser Ser Ser Pro Met Glu Tyr Phe Ser Ser Gly Leu 195 200 205
- Ile Leu Gly Ser Gly Gln Gln Thr His Phe Pro Ile Ser Thr Asn Ser 210 215
- His Pro Phe Ser Ser Ile Ser Asp His His His His Bro His His 225 230 235 240
- Gln His Gln Glu Phe Ser Phe Val Pro Asp His Leu Ile Ser Pro Ala 245 250 255
- Glu Ser Asn Gly Gly Ala Phe Asn Leu Asp Phe Asn Met Ser Thr Pro 260 265
- Ser Gly Ala Gly Ala Ala Val Ser Ala Ala Ser Gly Gly Phe Ser 275 280 285
- Gly Phe Asn Arg Gly Thr Leu Gln Ser Asn Ser Thr Asn Gln His Gln 290 295
- Ser Phe Leu Ala Asn Leu Gln Arg Phe Pro Thr Ser Glu Ser Gly Gly 305 310 315 320
- Gly Pro Gln Phe Leu Phe Gly Ala Leu Pro Ala Glu Asn His His His 325 330 335
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- Glu His Lys Gly Lys Gly Lys Asn 355 Asn

<210> 5

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CDS (70)	(81	6)												
5 gcat t	tcag	ctcc	a cc	gtag	gaaa	ctt	tctc	ttg	aaag	aaac	cc a	cago	aacaa	60
aaa at Me 1	tg tg et Cy	rs Gl	y G1	t go y Al 5	t at a Il	t at e Il	t to le Se	c ga er As	рту	r Al	c co a Pr	t ct o Le	c gtc u Val	111
g gcc s Ala	aag Lys	ggc Gly	cgt Arg 20	aaa Lys	ctc Leu	acg Thr	gct Ala	gag Glu 25	gaa Glu	ctc Leu	tgg Trp	tca Ser	gag Glu 30	159
t gct p Ala	tcc Ser	gcc Ala 35	gcc Ala	gac Asp	gac Asp	ttc Phe	tgg Trp 40	ggt Gly	ttc Phe	tat Tyr	tcc Ser	acc Thr 45	tcc Ser	207
c cat u His	ccc Pro 50	acc Thr	aac Asn	caa Gln	gtt Val	aac Asn 55	gtg Val	aaa Lys	gag Glu	gag Glu	gca Ala 60	gtg Val	aag Lys	255
g cag u Gln 65	gca Ala	aca Thr	gag Glu	ccg Pro	999 Gly 70	aaa Lys	cgg Arg	agg Arg	aag Lys	agg Arg 75	aag Lys	aat Asn	gtt Val	303
g Gly	ata Ile	cgt Arg	aag Lys	cgt Arg 85	cca Pro	tgg Trp	gga Gly	aaa Lys	tgg Trp 90	gcg Ala	gct Ala	gag Glu	att Ile	351
t cca p Pro	cga Arg	aaa Lys	ggt Gly 100	gtt Val	aga Arg	gtt Val	tgg Trp	ctt Leu 105	ggt Gly	acg Thr	ttc Phe	aac Asn	acg Thr 110	399
g gaa u Glu	gct Ala	gcc Ala 115	atg Met	gct Ala	tat Tyr	gat Asp	gtt Val 120	gcg Ala	gcc Ala	aag Lys	cag Gln	atc Ile 125	cgt Arg	447
t aaa p Lys	gcc Ala 130	aag Lys	ctc Leu	aac Asn	ttc Phe	Pro	Asp	ctg Leu	cac His	cat His	cct Pro 140	cct Pro	cct . Pro	· 495
n Tyr	Thr	cct Pro	ccg Pro	ccg Pro	tca Ser 150	tcg Ser	cca Pro	cga Arg	tca Ser	acc Thr 155	gat Asp	cag Gln	cct Pro	543
a Lys	aag Lys	gtc Val	tgc Cys	gtt Val 165	gtc Val	tct Ser	cag Gln	Ser	Glu	agc Ser	gag Glu	tta Leu	agt Ser	591
g agt o Ser	ttc Phe	ccg Pro	gtg Val 180	gag Glu	tgt Cys	ata Ile	gga Gly	ttt Phe 185	gga Gly	aat Asn	999 999	gac Asp	gag Glu 190	639
g aac In Asn	ctg Leu	agt Ser 195	tac Tyr	gga Gly	ttt Phe	gag Glu	ccg Pro 200	gat Asp	tat Tyr	gat Asp	ctg Leu	aaa Lys 205	cag Gln	687
a tcg le Ser	agc Ser 210	ttg Leu	gaa Glu	tcg Ser	ttc Phe	Leu	Glu	ctg Leu	gac Asp	ggt Gly	aac Asn 220	acg Thr	gcg Ala	735
ln Pro	Ser	cag Gln	ctt Leu	gat Asp	gag Glu 230	tcc Ser	gtt Val	tcc Ser	gag Glu	gtg Val 235	gat Asp	atg Met	tgg Trp	783
t gat eu Asp	gat Asp	gtc Val	att Ile	gcg Ala	tcg Ser	tat Tyr	gag Glu	taa	aag	aaaa	aaa	ataa	gtttaa	836
	CDS (70) G19 SgCat (1) G19 SgCat (CDS (70) . (81 G19 Sgcat ttcag aaa atg tg Met Cy 1 g gcc aag s Ala Lys t gct tcc p Ala Ser c cat ccc u His Pro g cag gca u Gln Ala a ggg ata g Gly Ile at cca cga p Pro Arg u Glu Ala at aaa gcc u Glu Ala at cca cga p Pro Arg ag gaa gct tt cca cga p Lys Ala 130 at tat act c Tyr Thr 145 cg aag aag ag Lys Lys co Ser Phe ag aac ctg an Asn Leu aa tcg agc act ccg agt accg agt	CDS (70) . (816) G19 Sgcat ttcagctco aaa atg tgt gg Met Cys G1 1 g gcc aag ggc s Ala Lys Gly t gct tcc gcc p Ala Ser Ala 35 c cat ccc acc u His Pro Thr 65 g ggg ata cgt ggly Ile Arg t cca cga aaa p Pro Arg Lys ag ggaa gct gcc au Glu Ala Ala 115 ag ggaa gct gcc u Glu Ala Ala 115 at caa gcc aag p Lys Ala Lys 130 at tat act cct an Tyr Thr Pro 145 cg aag aag gtc aag gct aag gcc aag aag gtc aac ccc aag aag gcc aag aag gtc aac ccc aag aag gcc aag aag ccg aag aag ccg aag aag ccg aag aag cag aac ccg agc cag accg accg	CDS (70)(816) G19 Sgcat ttcagctcca co aaa atg tgt ggc gg Met Cys Gly Gl 1 g gcc aag ggc cgt s Ala Lys Gly Arg 20 t gct tcc gcc gcc p Ala Ser Ala Ala Ala Ser Ala Ala Ala Ser Ala Ala Ala Gg gca aca gag u Gln Ala Thr Glu ag ggg ata cgt aag g Gly Ile Arg Lys ac cac cga aaa ggt p Pro Arg Lys Gly ac gag gca aca ggt u Glu Ala Ala Met 115 at caa gcc aag ctc p Lys Ala Lys Leu 130 at tat act cct ccg and Tyr Thr Pro Pro 145 ag agg agg gcd gcc tgc agg agg gcd gcc agg gca aca gct agg gca gcc acg agg gca aca ggt agg gca gcc acg agg gca aca gcc aca gcc acg ccc aca acc acc acc acc acc acc acc acc	CDS (70)(816) Sgcat ttcagctcca ccgtag aaa atg tgt ggc ggt gc Met Cys Gly Gly Al 1	CDS (70)(816) Sgcat ttcagctcca ccgtaggaaa aaa atg tgt ggc ggt gct at Met Cys Gly Gly Ala II 1	CDS (70)(816) G19 S gcat ttcagctcca ccgtaggaaa ctt aaa atg tgt ggc ggt gct att at Met Cys Gly Gly Ala Ile II 1	CDS (70)(816) G19 S gcat ttcagctcca ccgtaggaaa ctttctc aaa atg tgt ggc ggt gct att att tc Met Cys Gly Gly Ala Ile Ile Se 1	CDS (70)(816) G19 S Gcat ttcagctcca ccgtaggaaa ctttctcttg aaa atg tgt ggc ggt gct att att tcc ga Met Cys Gly Gly Ala Ile Ile Ser As 1	CDS (70)(816) G19 S gcat ttcagctcca ccgtaggaaa ctttctcttg aaag aaa atg tgt ggc ggt gct att att tcc gat ta Met Cys Gly Gly Ala Ile Ile Ser Asp Ty 1 S g gcc aag ggc cgt aaa ctc acg gct gag gaa s Ala Lys Gly Arg Lys Leu Thr Ala Glu Glu 25 t gct tcc gcc gcc gac gac ttc tgg ggt ttc p Ala Ser Ala Ala Asp Asp Phe Trp Gly Phe 35 c cat ccc acc aac caa gtt aac ggg aaa ggu His Pro Thr Asn Gln Val Asn Val Lys Glu 65 g cag gca aca gag ccg ggg aaa cgg agg aag u Gln Ala Thr Glu Pro Gly Lys Arg Arg Lys 65 g ggg ata cgt aag ggt ca tag ggg aaa cgg g gly Ile Arg Lys Arg Pro Trp Gly Lys Trp 85 g gag at cga aca ggt gtt aga gtt tgg ctt ggt gg ga gcd aca ggt gtt aga gtt tgg cgg tc ca cga aaa ggt gtt aga gtt tgg ctt ggt gg gaa gct gca gcc at ggc tat gat gtt gg gg ga gct gca gcc at ggc tat gat gtt gg gg ga gct gca gcc acg gct tat gat gtt gag tr cca cga aaa gct aac ttc ca gat cac lu Glu Ala Ala Met Ala Tyr Asp Val Ala Ala 115 at aaa gcc aag ctc aac ttc cca gat cac tr tat act cct ccg ccg tca tcg gca cac tr try Thr Pro Pro Pro Ser Ser Pro Arg Ser 145 146 147 148 149 140 150 151 165 167 168 169 169 160 160 170 180 180 180 180 180 180 18	CDS (70)(816) G19 5 gcat ttcagctcca ccgtaggaaa ctttctcttg aaagaaac aaa atg tgt ggc ggt gct att att tcc gat tat gc Met Cys Gly Gly Ala IIe IIe Ser Asp Tyr Al 1	CDS (700)(816) G19 5 Gcat ttcagctcca ccgtaggaaa ctttctcttg aaagaaaccc a aaa atg tgt ggc ggt gct att att tcc gat tat gcc cc Met Cys Gly Gly Ala Ile Ile Ser Asp Tyr Ala Pr 1	CDS (700)(816) G19 5 Gcat ttcagctcca ccgtaggaaa ctttctcttg aaagaaaccc acagc aaa atg tgt ggc ggt gct att att tcc gat tat gcc cct ct Met Cys Gly Gly Ala IIe IIe Ser Asp Tyr Ala Pro Le 1 1 g gcc aag ggc cgt aaa ctc acg gct gag gaa ctc tgg tca s Ala Lys Gly Arg Lys Leu Thr Ala Glu Glu Leu Trp Ser 20 t gct tcc gcc gcc gac gac ttc tgg ggt ttc tat tcc acc t gct tcc gcc gcc gac gac ttc tgg ggt ttc tat tcc acc t gct tcc gcc acc acc acg gct gag gaa ctc tgg ta 15 c cat ccc acc acc cac acc ggt aaa ggg gag ggc gtg this Pro Thr Asn Gln Val Asn Val Lys Glu Glu Ala Val 55 g cag gca aca gag ccg ggg aaa cgg aga gag aag a	CDS (700)(816) G19 5 gcat ttcagctcca ccgtaggaaa ctttctcttg aaagaaaccc acagcaacaa aaa atg tot ggc ggt gct att att tcc gat tat gcc cct ctc gtc Met cys Gly Gly Ala Ile Ile Ser Asp Tyr Ala Pro Leu Val 1

MBI15 Sequence Listing.ST25

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Gln Ala Thr Glu Pro Gly Lys Arg Arg Lys Arg Lys Asn Val Tyr Arg 65 70 75 80

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Pro Arg Lys Gly Val Arg Val Trp Leu Gly Thr Phe Asn Thr Ala Glu 100 105 110

Glu Ala Ala Met Ala Tyr Asp Val Ala Ala Lys Gln Ile Arg Gly Asp 115 120 125

Lys Ala Lys Leu Asn Phe Pro Asp Leu His His Pro Pro Pro Pro Asn 130 135 140

Tyr Thr Pro Pro Pro Ser Ser Pro Arg Ser Thr Asp Gln Pro Pro Ala 145 150 155 160

Lys Lys Val Cys Val Val Ser Gln Ser Glu Ser Glu Leu Ser Gln Pro 165 170 175

Ser Phe Pro Val Glu Cys Ile Gly Phe Gly Asn Gly Asp Glu Phe Gln 180 185 190

Asn Leu Ser Tyr Gly Phe Glu Pro Asp Tyr Asp Leu Lys Gln Gln Ile 195 200 205

Ser Ser Leu Glu Ser Phe Leu Glu Leu Asp Gly Asn Thr Ala Glu Gln 210 215 220

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MBI15 Sequence Listing.ST25 230

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Leu Leu Glu Glu Leu His Lys Gln Asp Glu Glu Arg Glu Val Phe Glu 145 150 155 160

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Gln Lys Thr Met Val Ser Phe Val Ser Gln Val Leu Glu Lys Pro Gly 180 185 190

Leu Ala Leu Asn Leu Ser Pro Cys Val Pro Glu Thr Asn Glu Arg Lys 195 200 205

Arg Arg Phe Pro Arg Ile Glu Phe Phe Pro Asp Glu Pro Met Leu Glu 210 215 220

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Ser Ser His Thr Arg Glu His Gln Val Glu Gln Leu Glu Ser Ser Ile 245 250 255

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			ttg gct tac gad Leu Ala Tyr Asp	
	Arg Gly Ser		ttg aat ttt ccg Leu Asn Phe Pro 205	Leu Arg
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Pro Val Tyr Gly 50	Arg Asn Pro	Ser Phe Ser	Lys Leu Tyr Pro 60	Cys Phe
Thr Glu Ser Trp	Gly Asp Leu 70	Pro Leu Lys	Glu Asn Asp Ser 75	Glu Asp 80

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Met Leu Val Tyr Gly Ile Leu Asn Asp Ala Phe His Gly Gly Trp Glu 85 90 95

MBI15 Sequence Listing.ST25

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Lys Trp Leu Lys Ser Gln Ser Leu Gln Asp Ala Lys Ser Ile Ser Pro

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Gln Cys Ser Ser Ala Pro Glu Ile Pro Arg Leu Phe Phe Ser Glu Trp 180 185 190

Leu Ser Ser Ser Tyr Pro His Thr Asp Tyr Ser Ser Glu Phe Thr Asp 195 200 205

Ser Lys His Ser Gln Ala Pro Asn Val Glu Glu Thr Leu Ser Ala Tyr 210 215 220

Glu Glu Met Gly Asp Val Asp Gln Phe His Tyr Asn Glu Met Met Ile 225 230 235 240

Asn Asn Ser Asn Trp Thr Leu Asn Asp Ile Val Phe Gly Ser Lys Cys 245 250 255

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402

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Phe Ser Ser Pro Glu Asp Leu Tyr Asp Asp Asp Phe Tyr Asp Asp Gln 50 55

Leu Pro Glu Lys Lys Arg Arg Leu Thr Thr Glu Gln Val His Leu Leu 65 70 75 80

Glu Lys Ser Phe Glu Thr Glu Asn Lys Leu Glu Pro Glu Arg Lys Thr 85 90 95

Gln Leu Ala Lys Lys Leu Gly Leu Gln Pro Arg Gln Val Ala Val Trp

Phe Gln Asn Arg Arg Ala Arg Trp Lys Thr Lys Gln Leu Glu Arg Asp 115 120 125

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Gln Val Pro Glu Pro Asn Gln Leu Asp Pro Val Tyr Ile Asn Ala Ala 180 185 190

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MBI15 Sequence Listing.ST25

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Lys Leu GIY Asn Ser Ser Asn Pro Asn Thr Cys GIY Asn GIY Asn 355 355 ggc agc tcc ctc ctc ctc ctc ctc tct ctc ctc ctc tta 1218 tcg gcg tct gat cgg atc gat cgg atc gat cgg tct gaa gag gat gag cat tca aag gag gag cat tca cac aac cat tat tcb gag cat tat tcb gag cat tcb lub lub <t< td=""><td>Leu</td><td>ttg Leu</td><td>gaa Glu</td><td>gag Glu</td><td>ttt Phe</td><td>Сув</td><td>agt Ser</td><td>gtt Val</td><td>gga Gly</td><td>aga Arg</td><td>Gly</td><td>ttt Phe</td><td>ttg Leu</td><td>aag Lys</td><td>aag Lys</td><td>Asn</td><td>1122</td></t<>	Leu	ttg Leu	gaa Glu	gag Glu	ttt Phe	Сув	agt Ser	gtt Val	gga Gly	aga Arg	Gly	ttt Phe	ttg Leu	aag Lys	aag Lys	Asn	1122
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Pro Gln Arg Gly Leu Pro Glu Arg Ser Val Asn Ile Leu Arg Ala Trp 505 Ctc ttc gaa cat ttc ctt cac ccg tat cca agt gat gca gat aaa cac Leu Phe Glu His Phe Leu His Pro Tyr Pro Ser Asp Ala Asp Lys His 520 Cta ttg gct cga cag act ggt tta tcc aga aat cag gta tca aat tgg Leu Leu Ala Arg Gln Thr Gly Leu Ser Arg Asn Gln Val Ser Asn Trp 545 ttc ata aat gct agg gtt cgt tta tgg aaa cca atg gtg gaa gaa atg Phe Ile Asn Ala Arg Val Arg Leu Trp Lys Pro Met Val Glu Glu Met	Gln					Gln					Glu					Arg	1602
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			acg Thr													1986
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Ser Asp Ser Pro Asn Leu Thr Thr Gln Gln Lys Gln Glu His Gln Arg Page 24

Val Glu Met Asp Glu Glu Ser Ser Val Ala Gly Gly Arg Ile Pro Val 65 70 75 80

Tyr Glu Ser Ala Gly Met Leu Ser Glu Met Phe Asn Phe Pro Gly Ser 85 90 95

Ser Gly Gly Arg Asp Leu Asp Leu Gly Gln Ser Phe Arg Ser Asn 100 105 110

Arg Gln Leu Leu Glu Glu Gln His Gln Asn Ile Pro Ala Met Asn Ala 115 120 125

Thr Asp Ser Ala Thr Ala Thr Ala Ala Ala Met Gln Leu Phe Leu Met 130 135 140

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- Gln Ser Cys Glu Leu Leu Gly Asp Lys Asp Ala Ala Gly Ile Ser Ser 450 455 460
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498

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Pro Pro Met Met Leu Gln Leu Gly Ser Gly Glu Gly Ser His Met 50 . Page 28

Ser Ala Ala Gly Leu Ser Gly Val Asp Gly Gly Leu Gly Gly Gly Ala 35 404045

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Pro Glu Gly Gly His Gly Ser Gly Lys Arg Phe Ser Asp Asp Val Val

Asp Asn Arg Cys Ser Ser Met Lys Pro Val Phe His Gly Gln Pro Met
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Glu Leu Val Pro Thr Val Asn Lys Thr Asp Arg Ala Ala Met Ile Asp 180 185 190

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Arg Thr Pro Gln Pro Ala Trp Glu Lys Trp Ser Asn Asp Gly Thr Glu 245 250 255

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MBI15 Sequence Listing.ST25

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Leu Pro Trp Lys Ala Lys Met Gly Glu Lys Glu Trp Tyr Phe Phe Cys 65 70 75 80

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680

728

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Ala Ala Leu Lys Phe Lys Gly Thr Lys Ala Lys Leu Asn Phe Pro Glu 85 90 95

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639

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180

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Leu Glu Lys Leu Gln Ser Arg Gln Ser Pro Glu Ile Glu Gln Thr Asp 95 $$

Ile Pro Ile Lys Lys Arg Lys Gln Asp Pro Asp Glu Phe Leu Gly Phe 100 105 110

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						cct Pro										295
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						atg Met										391
						ctc Leu										439
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						ctg Leu										631
						acc Thr										679
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						atg Met										775
						ggt Gly 250										823
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Tyr Ser Gly His Gly Ser Arg Gln Arg Asn Tyr Asn Gly Asp Glu Val 165 170 175	
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Gly Gly Glu Ala Ile Ser Ile Ser Gly Cys Asp Asp Asp Gln Thr Ser 260 265 270

Ala Asp Thr Ser Ala Leu Ser Lys Ile Thr Ser Thr Gly Ala Met Thr 275 280 285

Phe Cys Phe Ile Gln Ala Ile Glu Arg Ser Ala Gln Gly Thr Thr Tyr 290 295 300

Gly Ser Leu Leu Asn Ser Met Arg Thr Thr Ile Arg Asn Thr Gly Asn 305 310 315 320

Asp Gly Gly Gly Ser Gly Gly Val Val Thr Thr Val Leu Ser Met Leu 325 330 335

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MBI15 Sequence Listing.ST25

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Asp Thr Gly Gly Ser Lys Gly Leu Thr Gly Asp Leu Val Glu Lys Ile 165 170 175

Pro Lys Met Thr Ile Thr Gly Asn Asn Asn Thr Asp Ala Ser Glu Asn 180 185 190

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MBI15 Sequence Listing.ST25

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Lys Ala Ala Ser Leu Glu Asp Glu Val Ala Arg Leu Arg Ala Val Asn 115 120 125

Gln Gln Leu Val Lys Arg Leu Gln Asn Gln Ala Thr Leu Glu Ala Glu 130 135 140

Val Ser Arg Leu Lys Cys Leu Leu Val Asp Leu Arg Gly Arg Ile Asp 145 150 150 160

Gly Glu Ile Gly Ser Phe Pro Tyr Gln Lys Pro Met Ala Ala Asn Ile 165 170 175

Pro Ser Phe Ser His Met Met Asn Pro Cys Asn Val Gln Cys Asp Asp 180 185 190

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MBI15 Sequence Listing.ST25

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1302

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Lys Trp Glu Phe Leu Asn Asp Asp Phe Val Arg Gly Arg Pro Tyr Leu $85 \hspace{1.5cm} 90 \hspace{1.5cm} 95$

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Gly Lys Leu Gly Glu Arg Glu Thr Ser Asp Glu Gln Gly Glu Arg Ile 305 310 315 320

Pro Arg Leu Arg Tyr Leu Asp Gln Arg Leu Arg Gln Gln Arg Ala Leu 325 330 335

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MBI15 Sequence Listing.ST25

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35

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· raye /1	

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MBI15 Sequence Listing.ST25 335

Thr Ser Ser Asp Ala Ser Ala Glu Pro Leu Leu Thr Ser Ser Glu Glu 340 345 350

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. 325

INTERNATIONAL SEARCH REPORT

Interna al application No.
PCT/US00/31418

A. CLAS	SIFICATION OF SUBJECT MATTER		-	Į.								
IPC(7)	· A01H 1/00 5/00: C12N 5/14, 15/82			İ								
US CL	435/320 1 419, 468; 800/278, 279, 287, 301, 305-310, 312, 314, 317, 320, 322											
Assording to	International Patent Classification (IPC) or to both nat	ional clas	sification and IPC									
B. FIELI	DS SEARCHED											
			a'hala)									
Minimum doo U.S.: 43	Minimum documentation searched (classification system followed by classification symbols) U.S.: 435/320.1, 419, 468; 800/278, 279, 287, 301, 305-310, 312, 314, 317, 320, 322											
Documentation	on searched other than minimum documentation to the	extent tha	t such documents are included	in the fields searched								
Electronic da EAST, USPA	ta base consulted during the international search (name AT; STN, Agricola, CaPlus, Biosis, Embase	e of data t	pase and, where practicable, so	earch terms used)								
C. DOC	UMENTS CONSIDERED TO BE RELEVANT											
Category *	Citation of document, with indication, where app	ropriate,	of the relevant passages	Relevant to claim No.								
X	WO 97/47183 A1 (PURDUE RESEARCH FOUNDA	TION) 1	B December 1997	1-9, 12, 13, 25								
 Y	(18.12.1997), entire reference.			10, 11, 26, 27								
x	US 5,939,601 (KLESSIG et al) 17 August 1999 (17.0	08.1999),	entire reference.	1-9, 12, 13, 25								
 Y				10, 11, 26, 27								
A	Database Genbank on NCBI, US National Library o	f Medicin	e, (Bethesda, MD, USA)	1-13, 25-27								
	No. AB009055, SATO, S. et al 'Strucural analysis o IV. Sequence features of the regions of 1,456,315 bp	covered	by nineteen physically									
	assigfned P1 and TAC clones. 27 December 2000, D 41-54, see bases 16,003-16,490, 16,571-16,683 and	NA RES.	1998, Vol. 5, No. 1, pages									
	41-54, 300 0000 10,000 11,000											
	·											
				Ì								
Furthe	er documents are listed in the continuation of Box C.		See patent family annex.									
,	Special categories of cited documents:	*T*	later document published after the int date and not in conflict with the appli	ernational filing date or priority								
	ni defining the general state of the art which is not considered to be		principle or theory underlying the inv	rention								
	cular relevance Application or patent published on or after the international filing date	"X"	document of particular relevance; the considered novel or cannot be consid when the document is taken alone	ered to involve an inventive step								
ni " domine	nt which may throw doubts on priority claim(s) or which is cited to h the publication date of another citation or other special reason (as	-Y-	document of particular relevance; the	e claimed invention cannot be								
specifie	d) ·	-	considered to involve an inventive str combined with one or more other su	ep when the document is th documents, such combination								
1	nt referring to an oral disclosure, use, exhibition or other means	"&"	being obvious to a person skilled in to document member of the same pater									
priority	nt published prior to the international filing date but later than the date claimed											
Date of the	actual completion of the international search	Date of	mailing of the international se	earch report								
23 Februar	y 2001 (23.02.2001)	ļ	UT WHILE OF	701								
Name and	mailing address of the ISA/US	Authori	zed officer	RY J. DEY								
C	ornmissioner of Patents and Trademarks	David 1		AL SPECIALIST								
	ox PCT Vashington, D.C. 20231		Lientico									
	via (703)305-3330	Telepho	one No. 703-308- TECHNOLO	BY CENTER 1600								

Facsimile No. (703)305-3230
Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

Inte: nal application No.

PCT/US00/31418

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)
This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:
1. Claim Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
Claim Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. Claim Nos.: 14 because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)
This International Searching Authority found multiple inventions in this international application, as follows: Please See Continuation Sheet
 As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-13 & 25-27 and SEQ ID NOs 1&2
Remark on Protest The additional search fees were accompanied by the applicant's protest. No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Interior No.

PCT/US00/31418

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I-XXIX, claim(s) 1-14 and 25-27, drawn to a transgenic plant having modified seed characteristics, polynucleotides and vectors for producing said transgenic plant and a method of making said transgenic plant. Applicant must elect one pair of sequences (one nucleic acid and the corresponding amino acid translation) to be examined, i.e. SEQ ID NO: 1 and 2 in Group I, SEQ ID NO: 3 and 4 in Group II, SEQ ID NO: 5 and 6 in Group III, etc.

Group XXX, claim(s) 15-17, drawn to a method of identifying a factor that is modulated.

Group XXXI, claims(s) 18, drawn to a method of identifying a molecule that modulates activity or expression of a polynucleotide or polypeptide.

Group XXXII, claims(s) 19 and 20, drawn to an integrated computer system.

Group XXXIII, claim(s) 21-24, drawn to a method for identifying a polynucleotide sequence comprising selecting a nucleic acid sequence from a database that meets a selected sequence criteria.

The inventions listed as Groups I-XXXIII do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The inventions listed as Groups I-XXXIII do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Groups I-XXIX are drawn to a transgenic plant and a method of producing said plant with a nucleic acid sequence. The methods of Groups I-XXIX differ from each other in that they are directed to a plant transformation method and transgenic plant with a structurally and functionally distinct nucleic acid sequence which encodes a structurally and functionally distinct amino acid sequence. In addition, Groups XXX, XXXI and XXXIII are different methods from any of Groups I-XXIX in that they have different method steps and different end products, and Group XXXII requires a computer system. Thus, there is no single special technical feature, which links the inventions of Groups I-XXXIII under PCT Rule 13.2.